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SOIL SCIENCE

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Professor K. K. Gedroiz

The staff of the department of soil science of the New Jersey Agricultural Experiment Station, and of the College of Agriculture of Rutgers University have learned with keen sorrow and regret of the death of Prof. K. K. Gedroiz, and, with their colleagues at other institutions and other countries, recognize his passing as a most serious loss to science and agriculture. The young but rapidly growing science of the soil has lost in Professor Gedroiz an outstanding scholar whose contributions to our knowledge of the base exchange capacity and the colloidal properties of the soil have revolutionized our ideas concerning this important branch of soil science. As president of the Second International Soil Science Congress that convened in Russia in 1930, he contributed materially by his great reputation toward making the congress a success, even though ill health prevented him from taking a part in the deliberations of the congress. Russia has given many great men to soil science, and the name of Gedroiz will be remembered with those of Dokutschaiev, Sibirtzev, Kossowitch, Glinka, and many others, as having laid the foundation of a new science, which is at the very base of agriculture. The director and the members of the staff of the New Jersey Agricultural Experiment Station and of the College of Agriculture of Rutgers University wish to convey to the Academy of Sciences of the U. S. S. R. and to their colleagues in the Union and in other countries this message of sympathy and profound regret.

Academician Konstantin Kaetonovitch Gedroiz

1872–1932

Within the last five years, it has become the sad task of the writer to record the deaths of Russian scientific men in the field of soil science and plant nutrition. One after another, within this short period of time there passed away, first Glinka, then Omeliansky, Neustruev, Kostytchev, and now Gedroiz, who died from arteriosclerosis on October 5. All were brilliant investigators. All have made outstanding contributions to the new but rapidly growing science of the soil: Glinka and Neustruev, in the field of genesis and classification, Omeliansky and Kostytchev in the field of microbiology, and Gedroiz in the field of chemistry. None was more original and none has contributed more toward revolutionizing the prevailing ideas in soil science than Gedroiz.

Gedroiz was born in 1872 in Bender, Government of Bessarabia, the son of a physician. He was the next to the last child of a large family. Gedroiz can be considered truly international in nature, both by birth and education. His father was the son of a Lithuanian (father) and a Pole (mother); his mother was the daughter of a Hungarian (father) and a German (mother). His childhood was spent in peasant surroundings in the southern part of Bessarabia (now Roumania), amidst Bulgarians, Turks, Roumanians, Greeks, and Russian Raskolniks. He received his preliminary training at a Real school in Kiev and his higher education at the Forestry Institute, in St. Petersburg, from which he graduated in 1897. As a student, he became interested in science, utilizing the opportunity which was presented at the soil science laboratory of P. to L. Kossowitch of the Forestry Institute. His first investigation dealt with the solubility of the phosphoric acid of the Russian low grade phosphates in various solvents. The data obtained were utilized by Kossowitch for his work on the Russian low grade phosphates (Volume II of the Works of the Agricultural Chemistry Latoratory of the Agricultural Institute in St. Petersburg).

On graduation from the Forestry Institute, Gedroiz was invited by Kossowitch to assist him in the organization of the Agricultural Chemistry Laboratory of the Agricultural Institute. From that time until 1915 he served this institute, as scientific collaborator in the laboratory and in charge of the greenhouse experiments. He continued, simultaneously, his training at the University of St. Petersburg, in the Faculty of Natural Science and Mathematics and received his diploma in 1903. Beginning with 1900 he took an active part in the publication of the *Journal of Experimental Agronomy* under the editorship of Kossowitch, first as collaborator and assistant editor, and, when Kossowitch died (1915), as editor.

In 1908, Gedroiz undertook a series of scientific investigations at the Bureau

of Agriculture and Soil Science of the Scientific Commission of the Agricultural Institute. He continued his connection with this institute, which frequently changed its name, until 1928. His first investigations were the now famous studies of the soil colloidal complexes and the absorbing capacity of the soil. These resulted in a series of brilliant papers, which, unfortunately, as a result of the World War and the Russian revolution, remained unknown to the outside world until 1923, when a mimeographed edition of an English translation of a selected group of these papers was made available in America. In 1926, a summary of these papers was presented at the Conference of the Second Commission of the International Society of Soil Science in Groningen by Dr. Page. When the writer visited, in 1924, the famous German soil chemist Ramann, he found him deeply immersed in the study of Gedroiz's papers in the English translation, although Ramann himself knew some Russian.

In 1919, Gedroiz was elected by the faculty of the Leningrad Forestry Institute as professor of soil science. In 1927, he was elected correspondent of the Academy of Sciences of U. S. S. R. and was given the Lenin prize for his work. In 1929, the Academy of Sciences elected him an active member. In 1930, because of his election to active membership in the Russian Academy of Sciences, he gave up his active teaching, of which he was never very fond. In 1929, Gedroiz was elected director of the Soil Science Institute of the academy in the name of Dokutchaev. In addition to these positions, he also had charge, during this period of time, of the soil chemical laboratory of the Experimental Forestry Institute and of the laboratory of the Pedological Dokutchaev Commission (1916–1918); he lectured on soil science at the Leningrad Polytechnicum (1919–1921) and in the Leningrad Agronomical Institute (1918–1922). From 1921 to 1929, he took an active part in the Nossov Experimental Station, and at the time of his death he was an active member of the Dolgoprudnoe Experimental Field of the Scientific Institute of Fertilizers. Following the death of Professor Glinka, Gedroiz was unanimously elected in 1930 as president of the Second International Congress of Soil Science.

Gedroiz's scientific contributions comprise over 100 scientific papers, published largely in the *Journal of Experimental Agronomy* and in the *Contributions of the Agricultural Chemical Laboratory of the Agricultural Institute.* In addition to these, he wrote a book on chemical analysis of the soils, which was translated into German; at the time of his death, he was in the process of preparing the third Russian edition. He also wrote a monograph on "The Absorbing Properties of the Soil," which was also translated into German.

In 1931, the *Journal of Experimental Agronomy*, of which he had been editor since 1915, ceased publication. (Only a few issues had appeared at irregular intervals since 1926.) Its place was taken by a new journal, *Chemization of Socialistic Agriculture*, of which he was made one of the editors and an important contributor.

The investigations of Gedroiz deal primarily with the physical, physicochemical, and chemical properties of the soil. The origin of the soil and its

degradation; the formation of saline, alkaline, and acid soils; and classification of soils on the basis of their absorptive power, all received careful consideration and his contributions to this field are most outstanding. On the basis of these investigations, he has drawn a clear picture of the soil, especially the soil absorbing complex.

Personally, professor Gedroiz was very modest and unassuming. The civil war in Russia, following the revolution, has left a marked impress upon his family. With a brother killed in the revolution, one son killed in the war, another son maimed for life, the only daughter just having passed through an epidemic of typhus, he was still able to continue his work almost uninterruptedly except for the physical inconveniences and lack of sufficient supplies. When the writer of these lines was entertained at the home of Professor Gedroiz at the Forestry Institute in Leningrad in 1924, the writer expressed his great surprise at Gedroiz's ability to carry on under these circumstances (some of his best work appeared during that period). Professor Gedroiz replied with a smile: "We must adjust our lives, the scientific work must go on, at any cost." His wife was his constant companion and shared with him cheerfully all his difficulties.

During 1921 and 1922, Gedroiz carried out his studies under the most difficult and trying circumstances. Frequently the most essential pieces of apparatus and supplies were completely lacking and could not be procured at any price. Without complaint, however, he carried out his work.

His letters were always sincere and full of enthusiasm. The following few extracts from an extensive correspondence which lasted uninterruptedly for ten years, will serve better to illustrate the nature of the man and his devotion to his work and to his family than any amount of description.

June 17, 1923

"Dear Colleague:

"I am in receipt of your kind letter. Will be very glad if you will send me a copy of your translation of my papers; I will look it over, possibly change something here and there and supplement it further in accordance with my new experimental data. At present, the status of printing is at a very slow pace with us; my last papers, dealing with solonetzes, are awaiting their turn at the printers. As soon as they appear, I will send them to you.

"We are here in great need of American scientific literature. The *Journal of Experimental Agronomy* (*Zhurnal Opitnoi Agronomii*), of which I am editor, has ceased to receive, since 1918, American scientific literature, including the publications of the U. S. Department of Agriculture. We have only the first 4 volumes of *Soil Science*. The Journal has no money to subscribe for books and other journals. I can only offer an exchange arrangement; we have available the complete files of the Journal for 1909–1920, while the issue for 1921–1922 is being published now. I would be very thankful to you if you could help the *Journal of Experimental Agronomy* to receive the American journals

in the field of agriculture, soil science, plant nutrition, as well as the publications of various experiment stations. You could thus render a great service to Russian agronomists."

August 6, 1923

"I am in receipt of your letter of 12/VII and the translations of my three papers. I am very much indebted to you for your trouble concerning American scientific literature. I have glanced through the translations. I am not as good an English scholar as to be able to be a good judge, but I liked the translation; it seems to me quite accurate and one can read it readily. I have begun a number of investigations, but have not brought them as yet to a state where the results can be published. The conditions for scientific work are very difficult here: for example, since 1919 there has been no gas in Leningrad. We have to use kerosene or denatured alcohol, which is very expensive and inconvenient. One has to do everything himself, including dish washing. Conditions as they are force one to give up scientific work altogether and do something else. But by force of habit, established during a period of nearly 25 years, one does his work and cannot leave the laboratory in sipte of the fact that it ruins one's health, due to the difficult material conditions and the most unfavorable conditions for carrying out one's work."

February 9, 1924

"In regard to my last papers (sent in original copy, in an unpublished form), I believe it will be best to have them mimeographed (do not forget, please, to send me a copy of each). They are already set in type for the *Journal of Experimental Agronomy*, but it is quite impossible to say when they will be published, due to a lack of paper and money at the present time; possibly soon, and possibly only in a year from now or later. . . . . It is better to have them mimeographed. I am now completing my last paper, "Soil absorbing complex and the absorbed soil cations as a basis for a genetic soil classification." Possibly it will appear in print here, and possibly not! If not, I will then ask you to see whether it cannot be placed in *Soil Science*. The paper is of general interest. We may meet in Rome, if not—I hope to see you in our home in Leningrad."

March 9, 1924

"I am at present much overburdened with teaching duties at the Forestry Institute. There are a great many students who are striving to obtain knowledge of the soil; the conditions are not sufficiently favorable, however, to satisfy these growing interests. One has to spend, therefore, much time in preparation. I shall hardly get to Rome. The chief difficulty lies in the fact that my

son is very sick, for nearly a year now; the nature of the disease is such that it is almost impossible for me to be away. I do hope that we will meet in Leningrad . . . ."

January 6, 1925

" . . . . My work does not progress very well. One has to devote altogether too much time to teaching students, and due to their large numbers and very limited facilities and means available for teaching, it is not such an easy problem, especially since I am not primarily a teacher by nature. However, without a professorship, it is impossible to live and devote oneself only to scientific work. Since materially our condition is also not of the very best, the purely scientific investigations are very much hampered. The monograph concerning the solonetzes is far from progressing, due to above circumstances. However, my new system of soil classification is already published, and will arrive within a few days from the printers. It has an extensive summary in German, but I would like very much to see it published in English. It forms in a way a completed philosophy of all my previous work. Perhaps Dr. Scofield will be interested in having it mimeographed, if it cannot be printed . . . . "

July 27, 1925

"Your letter of the 29/VI did not reach me in Leningrad but was forwarded to me to the Nossov Experimental Station (Government of Tchernigov) where I am spending the time now partly for the purpose of resting and partly for continuing my investigations, especially in order to keep in touch with the soil. I hope very much that I shall be able to come to America in 1927, to see and become acquainted with American life and work. I am planning to begin this winter to study English, in order to understand the language and make myself understood."

April 29, 1926

" . . . . Concerning my life, there is nothing to boast of. My son is in a very critical condition; it seems that his case is hopeless. My daughter is not well so that it will be necessary to go south during the summer, probably to Crimea. During a period of nearly five years, when they were both at an age of transition, dangerous for their health, they experienced want which is showing now. Even now, unfortunately, the living conditions are not sufficiently favorable, so that one can hardly expect to catch up with the former losses.

"I believe, however, that during the summer, when and if I have had a chance to rest up a little, I shall prepare a complete paper on the origin of alkali soils and will send it to you for *Soil Science* . . . ."

From a later letter:

"I began to apply for the necessary permission to come to your country for a visit; I would very much like to see your country and visit your institutions. I am only somewhat perplexed by the language difficulties. It is not very easy to learn to speak and carry on a conversation. I have always experienced language difficulties, in spite of the fact that I studied foreign languages and visited the countries of Germany and France. I learn to read very easily and quickly, but to speak is another matter.

"My investigations on the physical properties of the soil are being continued. The results obtained are highly interesting; unfortunately, the time available for this work is very limited, and I do not believe that I will be able to complete this work in time to be able to present it at the meetings of the commission in Groningen. I believe that I will be able to explain definitely this type of soil absorption, as I have done for the exchange capacity of absorption (physico-chemical). I am also beginning to outline an investigation of the mechanical absorptive power of soils (retention by soils of solid particles) . . . ."

November 18, 1926

" . . . . I am studying the English language; the progress is not as rapid as I was hoping; the time that I am able to give to it is very limited. I hope, however, that I will soon be able to write to you in English. I am afraid, however, that I will not master the pronunciation; it seems though that English comes to me much easier than German and French.

"I am waiting impatiently for the end of December when I shall be able to leave Leningrad and go to Nossovka, even if only for a couple of weeks. Only there is it possible to do the type of scientific work which resembles, even if not fully, to some extent at least, the type of work that I have done previously. Here, however, in Leningrad, one is merely pouring from one empty barrel into another; only the nerves become exhausted, and the work does not progress very far. Constantly, one is busy with all sorts of things which have nothing to do with scientific investigations. In Nossovka things are different, even if I am able to spend there twice a year only 2–4 weeks, since I have nothing to do there with administrative work. Of course if I lived there everything there would naturally be the same as here.

"As usual, I have again to ask some favor of you. I have sent to Dr. Al. Howard of the Agricultural Research Institut at Pusa my publications. The package was accepted here but was returned from abroad; nobody knows the reason. I am sending this package to you, and would ask you to forward it to Howard. I want you to excuse me, but, as you see, we Russians are totally helpless in many respects against the outside world. I have sent a registered letter to Howard; if that comes back, I will again appeal to you. . . . .

"Geetings from my wife. My daughter is improving in Crimea. I do not know, however, how the Leningrad climate will affect her. My son is in a bad condition."

May 15, 1927

" . . . . I am very sorry to say that I will not be able to see you again this year, since I am not going to the congress. I am extremely tired, my family affairs are not of the pleasantest; some of the investigations begun at Nossovka have to be completed; these are some of the causes which prevent me from leaving Russia. There is also another cause which has influenced my decision: the number of candidates that can go to the congress is small, while the number of those that desire to go is very large. As in all such cases, it is difficult to make a fair division, which would remove every cause of misunderstanding. In all such instances, and in this case as well, I try to step aside.

"I have certain hopes, however, of being able to visit America in the very near future, possibly even next year. I will then be able to see the things that I am interested in and not what will be shown to me."

November 15, 1927

" . . . . K. D. Glinka died. He came back from America a sick man. It seems that he died from a swelling of the lungs. All our pedologists that have been to the congress have returned completely exhausted, but they were all younger and stronger so that they soon began to improve."

Early in 1928

"I have decided not to go abroad—my health does not permit, I expect to spend the whole summer in Nossovka and partly near Kiev, but do not as yet know exactly where. If you plan to come to Leningrad during the summer, I want to offer you my apartment—it is quite empty, but is looked after by the wife of one of the workers at the Pedological Institute; you can use her services. I am living now in another building, also a part of the Institute."

Kislovodsk, December 29, 1929

"I was forced to go at the beginning of December to Kislovodsk, since I felt very badly, as a result of extreme nervous exhaustion and weakening of the heart activity. I am here together with my wife and am trying to recuperate. I am forbidden every form of mental work. It looks as if several months will be required."

March 26, 1930

"I am returning within a few days to Leningrad and will begin my investigations anew. I have improved considerably in Kislovodsk, but my heart is

still weak, due to arteriosclerosis. It is quite possible that this will prevent me from taking an active part in the soil congress. My sudden leave last fall from Leningrad prevented me from completing a series of investigations on the action of hydrogen peroxide upon the soil, and from preparing for publication a paper on the influence upon plant growth of the exchangeable cations which saturate a soil. I have completed here an article of 45 pages, in which I made clear that in acid soils, both natural and those that have been made acid by the use of artificial fertilizers, there is no exchangeable aluminum, but only adsorbed hydrogen ions. Is there a possibility of publishing it in *Soil Science*? If I do not come to the congress, I hope to have an opportunity of seeing you privately."

September 25, 1930

"I am awfully sorry that I could not see you and several other colleagues during the congress. My health has become so much worse that I was ordered to keep absolutey quiet, preferably in one place and avoid trips and visits. New impulses and any physical work cause terrific pain in my heart and prevent me from working for some time. In a peaceful state, I can continue my work. I, therefore, decided to move to Moscow where there exists an experimental field (Dolgoprudnoe) as a part of the Institute of Fertilizers. I am there in charge of the agro-chemical division. A large laboratory is being built here for that purpose. Meanwhile, I use the smaller laboratories connected with the station. I should like to have your candid opinion concerning the congress, its organization and whether the foreigners were satisfied."

December 14, 1931

"My work is progressing very slowly. Conditions in Dolgoprudnoe are still unfavorable; we already have a good greenhouse, and I have been able to utilize it in my investigations on the rôle of exchangeable Ca and Mg in plant nutrition, but the chemical facilities are still poor, since the laboratory is not completed as yet. My health is poor. I have to stay in Dolgoprudnoe and not leave it. Every trip causes terrible pain. I am now spending much time in preparing the third edition of my books, 'Chemical Soil Analysis' and 'The Absorptive Capacity of the Soil.' I would like very much to see you and talk over a great many things. . . . . "

Finally from the last letter, dated September 9, 1932:

"Dear Colleague!

"How are you and where are you? Have not heard from you since last spring. I am planning to undergo a cure again. Do not know as yet where, possibly in Caucasia. Beginning last July, my health has been failing. I am sending you the last 3 issues, namely 5, 6, and 7 of our new journal (*Chemization of Socialistic Agriculture*).

"I carried through this year a large vegetation experiment, where I have shown definitely that an antagonism exists between adsorbed calcium and magnesium. The injurious effect of exchanged magnesum in the field can be neutralized by the introduction into the absorbing complex of calcium, and vice versa. Upon leaving for the south, I will write to you my new address. With best greetings from all of us,

Yours,
K. Gedroiz"

On October 10, a telegram was received from Professor Jarilov, vice-president of the International Society of Soil Science, which reads as follows:

"Fifth October deceased President Second Congress International Society Soil Science, Member Academy of Sciences, Professor Gedroiz."

So has passed a man whose whole life was devoted to the science of the soil. The future historian of this science will give an honorary place to this brilliant and indefatigable investigator.

List of publications of Professor Gedroiz dealing with base exchange and soil absorption

1908. Colloidal chemistry and soil science. *Zhurnal Opitnoi Agronomii (Journal Experimental Agronomy)* 9: 272.

1911. What soils are acted upon by low grade phosphates? Soils saturated and unsaturated with bases. *Zhur. Opit. Agron.* 12: 529.

1912. Colloidal chemistry as related to soil science. I. Colloidal substances in the soil solution. Formation of sodium carbonate in the soil. Alkali soils and saline soils. *Zhur. Opit. Agron.* 13: 363.

1914. Colloidal chemistry as related to soil science. II. Rapidity of reaction exchange in the soil. The colloidal condition of the soil saturated with various bases. The indicator method of determining the colloidal content of the soil. *Zhur. Opit. Agron.* 15: 181.

1915. The action of electrolytes on clay suspensions. Communication 24 from the Bureau of Agriculture and Soil Science of the Scientific Committee of the Main Department of Land Organisation and Agriculture, Petrograd, 1915.

1916. The absorbing capacity of the soil and the zeolitic bases of the soil. *Zhur. Opit. Agron.* 17: 472.

1917. Saline soils and their improvement. *Zhur. Opit. Agron.* 18: 122.

1918. Contribution to the method of determining zeolitic bases in the soil. *Zhur. Opit. Agron.* 19: 226.

1918. Contributions to our knowledge of the absorptive capacity of soils. I. Rapidity of absorption, volume of absorption, and energy of absorption and replacement. *Zhur. Opit. Agron.* 19: 269; 20: 31.

1922. On the absorptive power of soils. Editorial Committee of the People's Commissariat of Agriculture, Petrograd, 1922.

1922. Soils unsaturated with bases. A method of determining in soils the hydrogen present in an absorbed condition. Soil requirement of lime as a neutralizing agent. *Zhur. Opit. Agron.* (1924) 22: 3.

1922. The hydrochloric acid determination of cations in soils present in an adsorbed condition. *Zhur. Opit. Agron.* 22: 53.

1922. Ultra-mechanical composition of soils and its dependence on the nature of the cations present in the soil in an absorbed condition. Liming as a measure of improving the ultra-mechanical composition of the soil. *Zhur. Opit. Agron.* 22: 29.

1925. The absorbing soil complex and the absorbed soil cations as a basis for the genetic classification of soils. Nossov Agricultural Experiment Station, Agricultural Division, Paper No. 38, Leningrad.
1926. Solodization of soils. Nossov Agricultural Experiment Station.
1926. Soil as a culture medium for the growth of agricultural crops. Soil colloids and soil alkalinity. Nossov Agricultural Experiment Station.
1926. On the soil structure and its agricultural significance. *Institute of Experimental Agronomy* (3).
1928. Alkaline soils, their origin, properties and melioration. Nossov Agricultural Experiment Station.
1930. On the question of exchangeable hydrogen and on the exchangeable aluminum in acid soils. *Potchvoviedienie*. 25(5): 5.
1930. Exchangeable cations of the soil and the plant: I. Relation of plant to certain cations fully saturating the soil exchange capacity. *Soil Sci.* 32: 51.
1931. Exchangeable cations of the soil and the plant: II. Liming of soil and the relation between exchangeable calcium and magnesium in soil. Udobrienie i urozshai. No. 11, p. 1047.
1931. Action of hydrogen peroxide upon the soil. Udobrienie i urozshai. Nos. 9 and 10.
1932. The soil absorbing complex, the plant, fertilization and melioration. Chemization of Socialistic Agriculture, No. 1.
1932. The absorbing capacity of the soil. Third edition, corrected and improved. 202 pages. Selkolchoziz.

SELMAN A. WAKSMAN,
New Jersey Agricultural Experiment Station,
New Brunswick, New Jersey

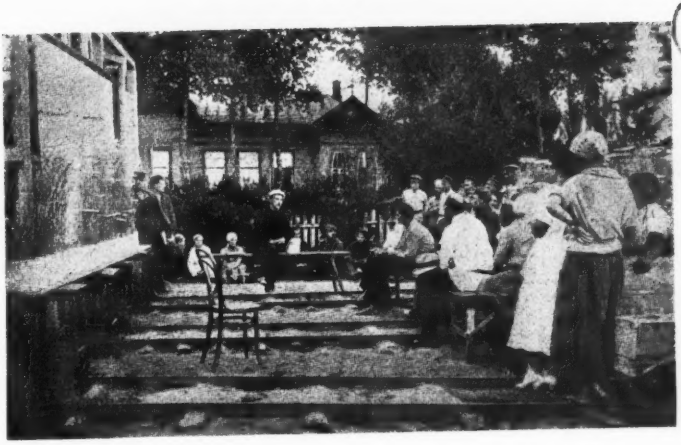
PLATE 1

PROFESSOR K. K. GEDROIZ

FIG. 1. Professor Gedroiz, just preceding the World War.

FIG. 2. Professor Gedroiz lecturing at the Nossov Experiment Station on the value of vegetation experiments.

FIG. 1



UNIV. OF MICH.

FIG. 2

THE DETERMINATION OF EFFECTIVE STRAINS OF RHIZOBIUM TRIFOLII DANGEARD, THE ROOT NODULE BACTERIA OF CLOVER, UNDER BACTERIOLOGICALLY CONTROLLED CONDITIONS[1]

DOROTHEA L. KEENEY[2]

Syracuse University

Received for publication May 17, 1932

The practices of crop rotation, and of ploughing under a leguminous crop, to add a larger amount of available nitrogen to the soil, date far back in agricultural history. The fact that the nitrogen content of the legumes could be increased by inoculation of soil or seed with certain bacteria, which incite the formation of nodules on the roots of the plants and fix atmospheric nitrogen in an available form, is a comparatively recent discovery,—the first recorded experiment establishing this fact having been performed in 1887.

In 1914, it became evident that many characteristic differences were associated with and restricted to the development of any one organism from certain well-defined groups of plants.

By 1926, the knowledge in this field had been extended by Fred, Whiting, and Hastings (23) in a summary of 10 years' study to include eight cross-inoculation groups, whereas Burrill and Hansen (11) distinguished eleven groups, within which species of the host plant are interchangeable for the purpose of inoculation and beyond which nodules cannot be produced. In 1928, Walker (52) listed 18 such groups, 7 of which contained more than one species of host plant, and the other 11, with one host only, were reported as incapable of cross-inoculation with other legumes so far as tested.

During the last decade interest has centered in the discovery of physiological differences in *strains within a single cross-inoculation group*. The distinctions are based on constant differences in the effects of inoculation on the plant, distribution of nodules over the root system, varying amounts of increased yield and nitrogen content when inoculated with different strains, and finally serological reactions with the organism as antigen.

The last named distinction, being of high specificity, is therefore of the greatest value. To date, six of the cross-inoculation groups have been subdivided by Fred, Whiting, and Hastings (23) on the basis of agglutination tests into from two to four agglutination groups, including strains of the organism which show other similar characteristics, such as variation in the power of nitrogen fixation.

In 1929, Baldwin and Fred (6) published an abstract of some of their current investigations on strain variation in *Rhizobium trifolii* in which 18 strains were tested for efficiency in aiding

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plant growth. Considerable variation was shown in the benefit derived by the host. An interesting variation in nodulation was coordinate with their effectiveness, namely, that more but smaller nodules were formed by the poor strains than the good, and they were distributed over the whole root system. In the good strains, the nodules were fewer, larger, and located on the upper portion of the root system.

Thus, at present, attention is directed toward a study of the efficiency of these distinct strains. Dunham and Baldwin (16), in 1931, tested the effect of double infection with good and poor strains and found that plants already infected with one strain of organism resist the entrance of a contrasting strain to a much greater extent than do nodule-free plants.

It is interesting to note that in most of the work done, an open pot method of cultivating the inoculated host plants has been used. In other experiments small seeds, e.g., clover and alfalfa, have been inoculated and grown on an agar substrate in test tubes, and Wilson, Hopkins, and Fred (55), in 1930, studied the fixation of nitrogen by plants grown on agar in 12-oz. and 32-oz. bottles. Care was exercised to reduce as far as possible the effect of factors detrimental to the physiology of the plant. However, since growth in closed containers, in agar, forces a highly artificial environment upon both the host plants and the invading bacteria, the authors themselves state that it is apparent "that this method is of little value in the comparison of the relative efficiency of strains except in a gross way."

It seemed desirable to test the effectiveness of various strains of *Rhizobium trifolii* under bacteriologically controlled conditions, imitating as nearly as possible the conditions found in nature, especially as they affect root development. To this end various strains were grown on plants of *Trifolium pratense* and *T. hybridum* in sterile sand cultures by a method, the details of which are described later. The ultimate purpose was to obtain tested strains of high efficiency in nitrogen fixation.

EXPERIMENTAL

History of Cultures

Twenty-two strains of *Rhizobium trifolii* were used in these investigations, seven of which were isolated locally and fifteen of which were "imported strains," obtained in culture from investigators in other universities or state experiment stations. Table 1 gives their history, Roman numerals having been used to indicate local strains and Arabic numerals imported strains.

All cultures have been grown on mannite agar with transfers approximately once a month. The formula for this medium is as follows:

Mannitol	20 gm.
Magnesium sulfate	0.2 gm.
Di-potassium phosphate	0.2 gm.
Sodium chloride	0.2 gm.
Calcium carbonate	5.0 gm.
Agar	15.0 gm.
Distilled water	1,000.0 cc.

Cultural Characteristics

The cultures show characteristic differences in growth on mannite agar slants of 2-day-old cultures, incubated at 27°C. The most pronounced differences

TABLE 1

History of cultures used in experiments

CULTURE NUMBER	SOURCE OF STRAIN	DATE OF ISOLATION
	Local strains	
I	Isolated from Trifolium pratense by Plant Physiology Class in Syracuse University, "Clover I"	Nov. 1927
II	Isolated from Trifolium pratense by Plant Physiology Class in Syracuse University, "Clover II"	Nov. 1927
III	Isolated by author from *T. pratense* collected near East Liverpool, N. Y.	Aug. 1928
IV	Isolated by author from *T. hybridum* collected near East Liverpool, N. Y.	Aug. 1928
V	Isolated by author from *T. repens* collected at Skaneateles, N. Y.	Aug. 1928
VI	Isolated from *T. pratense* by Josiah Lowe, Syracuse University	Nov. 1928
IX	Isolated by author from *T. hybridum*, Tully, N. Y. (growing in presumably acid soil)	Oct. 1928
	Imported strains	
1	"Red Clover 104W" Columbia, Mo. Isolated from exchange culture from University of Wisconsin. Represents five transfers	Mar. 1928
2	"Red Clover 105" Columbia, Mo. Isolated from exchange culture from U. S. D. A.-Leonard. Represents 29 transfers	Mar. 1925
3	"Red Clover 106" Columbia, Mo. Isolated from nodule. Represents 11 transfers	May 1927
4	Isolated from sand cultures supplied by Cornell Exp. Sta., Ithaca, N. Y.	Fall 1928
5	"Trifolium 1" Madison, Wis. Isolated from plants of *T. suaveolens*. Seed obtained in Germany and grown on new land. Tested 1927	Fall 1927
6	"Trifolium 203" Madison, Wis. Isolated from nodules of *T. pratense* at Madison. Tested 1921 and 1922. Always positive	1921
7	"Trifolium 205" Madison, Wis. From nodogen culture in 1922. Replated and tested annually 1922–1927. Always positive	1922
8	"Pa. Red Clover" from Penn. State College, pure culture	Transferred Syracuse Aug., 1928
9	"Trifolium #1" Mass. Agr. Col., Amherst, Mass.	Received in Syracuse Sept. 1928
10	"Trifolium #2," Amherst, Mass.	ditto
11	"A. Dickinson Co. Nod-o-gen Clover," Amherst, Mass.	ditto
12	"Mulford Co. 1927 Clover," Amherst, Mass.	ditto
13	"Earp-Thomas Co. Homogerm-Clover," Amherst, Mass.	ditto
14	"Earp-Thomas Co. Farmogerm-Clover," Amherst, Mass.	ditto
15	"*Rhizobium radicicola* on clover," Dept. of Bacteriology, Univ. of Nanking, Nanking, China	Spring 1930

occur in the abundance of growth and the consistency, since there is decided variation in gum production.

Tests for Purity of Cultures

All cultures were tested for purity before further experimentation was undertaken. The two methods used were those followed by Löhnis and Hansen (41) for distinguishing "*B. radicicola*" from the most frequent contaminator *B. radiobacter*. The substrata used were milk, and plugs of white potato cut with a slanting surface, sterilized in the autoclave for 15 minutes at 15 pounds pressure. They were then inoculated with a loop of material from the stock cultures.

If the culture tested is "*B. radicicola*," there will appear at the surface of the milk after 1 to 4 weeks a perfectly clear serum zone 2–5 mm. thick, while the milk below remains nearly unchanged. *B. radiobacter* forms first a slime ring on top and later the whole milk turns brown. On potato, "*B. radicicola*" forms a meager, transparent, slimy growth, whereas if *B. radiobacter* is present, the potato turns gray frequently and the growth is at first gray but later becomes a coli-brown slimy layer, thus making a sharp distinction between *B. radiobacter* and the nodule bacteria. This growth in milk and on potato is stated to be quite characteristic and can be used to great advantage as a test for purity of culture. The results of these tests showed that five of the cultures; namely, III, IV, 9, 12, and 13, did not give the characteristic reaction for "*B. radicicola*."

Tests for Positivity

All cultures were then tested for their ability to produce nodules on the host plant. As a growth medium 0.65 per cent agar in tap water was used in test tubes. For inoculum a bacterial suspension from 4-day-old cultures growing on mannite agar slants was made by washing the growth from the agar surface.

Inasmuch as more than half of the cultures had been isolated from red clover, seeds of *Trifolium pratense* were used. These were sterilized by immersion in 1:1,000 mercuric chloride, then thoroughly washed in sterile distilled water, and transferred to the culture tubes with a sterile platinum loop. Inoculation was obtained by adding five drops of the heavy bacterial suspension after the seeds had been placed in the culture tube. The cultures were kept in the dark for 2 or 3 days until the seeds had germinated, then taken to the greenhouse where they were set in sawdust up to the agar level to shield the roots against light. When the cultures were grown in summer, cheesecloth was used to cover and shade them from brilliant sunlight.

Plate 1, figure 1, shows the results of such tests for nodule formation.

Eight cultures showed no nodule formation, III, IV, 4, 5, 11, 12, 13, and 15. The others were all positive and produced abundant nodules in this and in all subsequent tests.

Following the test made in July, 1930, nodules were removed from each of

the positive cultures and the organisms reisolated, the original stock cultures being replaced.

Sand Cultures

A total of 10 sets of clover crops were grown in sand cultures, each crop growing for a period of 70–120 days before being harvested and tested. Two methods of setting up the sand cultures were used, open pot and bacteriologically controlled.

Open Pot Cultures.—The open pot method was used in the first three sets only. It is by this method that the majority of previous investigators have carried out their experiments. It consisted in the preparation of 1-gallon earthenware jars as culture chambers. These were filled with glacial sand which was very low in nitrogen content. The pH of the sand used was 8.2–8.4.

It was determined that 400 cc. of water per jar was necessary to bring the sand to the proper moisture content for germination and growth. This was added before sterilization, and heavy paper was tied tightly over the top of each jar. They were then autoclaved at 15 pounds pressure for 18 hours—6 hours for each of three consecutive days. After removal from the autoclave, they were cooled to room temperature before the seeds were planted. If the surface of the sand appeared dry after sterilization, sterile distilled water was added as required.

Viable red clover seeds, *Trifolium pratense*, were used. Sterilization of seeds was by immersion in 1:1,000 mercuric chloride as previously described. The same number of seeds was placed on the surface of each jar and it was immediately inoculated by pouring over it a bacterial suspension made from 4-day-old mannite agar cultures of one of the strains of *Rhizobium trifolii*.

The jars were then taken to the greenhouse and kept in a separate section where every precaution was taken to guard against contamination.

A sterile nutrient solution was prepared according to the formula used by Helz, Baldwin and Fred (31) as a "Modification of Bryan's." The following salts were ground together in a mortar to obtain a homogeneous mixture.

	gm.
Potassium chloride	10
Calcium sulfate	2.5
Magnesium sulfate	2.5
Calcium phosphate	2.5
Iron phosphate	2.5
Potassium phosphate, tribasic	2.5

The nutrient solution was made up on the basis of 3 gm. of mixed salts per liter, and 100 cc. of the solution was added to every jar every 2 weeks. If more moisture was needed, the plants were watered with distilled water only.

Sets I, II, and III each contained 22 such cultures, with 2–5 checks per set, i.e. cultures prepared in the same way except that they were not inoculated.

In harvesting the crops, the plants were carefully washed free of sand—sur-

plus water being removed—, placed in paper bags, and labelled. These were thoroughly dried in an oven kept at 90°C. for 24 hours, then at 105°C. for several days. Then each crop was reduced to a fine powder and placed in a tightly stoppered bottle.

Before the entire crop was weighed, the powdered material was again placed in an electric oven kept at constant temperature of 105°C. for a day, allowed to cool in a Frühling-Schultz desiccator, and then weighed.

For determination of the nitrogen content a modification of the modified Koch-McMeekin method (28) for micro-Kjeldahl tests was used. A 20-mgm. sample of the dried clover powder was placed in a chemically clean and dry Pyrex test tube graduated to 50 cc. To this was added for digestion 2 cc. of sulfuric acid, 1:1 dilution + 5 per cent copper sulfate, and the mixture boiled vigorously over a micro-burner until, after blackening, the characteristic dense white fumes began to fill the tube. At this time a watch glass was placed over the mouth of the tube and the flame was immediately reduced to an amount just sufficient to keep the mixture boiling gently for 5 minutes. To reduce the time necessary for digestion a few drops of Superoxol were then added, the burner having been moved aside so that the acid was not actively boiling. Better results are obtained if the Superoxol is dropped directly into the hot solution from a capillary pipette and not allowed to run down the side of the tube. Enough Superoxol is necessary to change the color of the mixture from dark brown to colorless or a light straw-color,—thus acting as a strong catalyst. Again the material is boiled gently for 5 minutes. If the solution discolors, an additional drop or two of Superoxol will clear it. After cooling, approximately 5 cc. of distilled water was added to the colorless solution and it was ready for distillation.

After the work of nitrogen determinations had been completed, it was noted that Sullivan and Horat (49), in 1929, had suggested that dry plant material may be digested in a test tube with the aid of Superoxol and the resulting analysis be of as high accuracy as the regular Kjeldahl method. In their microchemical tests, however, they used repeated additions of Superoxol, five drops at a time, and after the mixture cleared, boiling for 3 hours to complete digestion. An aeration process requiring 2 hours followed before the material was ready for titration.

In the method used in this investigation from 5–20 drops of Superoxol was added after the first 5-minute period of boiling, the number of drops depending on the amount necessary to clear the mixture, and thereafter digestion was completed with boiling for only 5 minutes more. The subsequent distillation required about 5 minutes, thus making the entire process previous to Nesslerization, a matter of about 20 minutes for each sample.

At this time the total plant nitrogen has been digested and changed to ammonium sulfate. The Koch-McMeekin method calls for direct Nesslerization but with the samples of clover material, this frequently resulted in a cloudy liquid which could not be used for comparison against a standard in a

colorimeter. Therefore it was found necessary to distill over the nitrogen into sulfuric acid.

Distillation consisted in first neutralizing the material by addition of a sufficient amount of a concentrated solution of sodium hydroxide, (410 gm. to 1 liter). The test tube had previously been connected with a small Liebig condenser which led into a 50-cc. volumetric flask containing 5 cc. of .1 *N* sulfuric acid and 10 cc. of distilled water. On the addition of the sodium hydroxide, the ammonium sulfate is changed to ammonium hydroxide, and when the test tube is heated with a microburner this becomes volatile and is distilled over into the flask containing the acid, where it again becomes ammonium sulfate, but all colloidal matter has been left behind and the liquid on Nesslerization remains crystal-clear. The heating usually requires from 3 to 5 minutes before crystals begin to form in the tube, which indicates the end point.

Figure 1 shows the arrangement of the apparatus used for distillation.

The material in the flask is then Nesslerized by the slow addition of 10 cc. of the Koch-McMeekin Nessler's solution, with constant shaking, and the mixture made up to 50 cc. with distilled water. It is then ready to be colorimetrically compared with a standard.

The standard is made up by adding to 5 cc. of ammonium sulfate, adjusted to contain 0.3 mgm. nitrogen in 5 cc., approximately 10 cc. distilled water, then 10 cc. Nessler's, and enough more water to make it up to 50 cc. A single standard can be used safely for readings for half an hour only, during which time a number of unknowns may be compared with it.

The Klett bio-colorimeter was used for these determinations and from the readings obtained, the milligrams of nitrogen and the percentages of nitrogen were calculated. Duplicate tests were run for each culture and the average of five colorimeter readings was taken for each sample. If there was an appreciable discrepancy in the average readings of the two samples, one or more samples were retested before further calculations were made.

Bacteriologically Controlled Cultures.—After a study of the results of sets I, II, and III it became obvious that the open-pot method was not satisfactory for a study of the effects of different strains of bacteria, since nodules were found on the roots of all cultures, including controls as well as the ones inoculated with strains which were non-nodule-producing in agar culture. The open-pot method was therefore abandoned, and it became necessary to devise a method which would supply bacteriologically controlled conditions, and at the same time be as nearly similar to natural conditions as possible.

In the last seven sets a modification of the apparatus suggested by Deatrick (15) was used, which in turn included an adaptation of an arrangement for keeping seeds sterile, suggested by Fred (20). The method makes it possible to supply sterile nutrient solution as required and still keep the jar covered and the plants protected against contamination.

Figure 2 illustrates the arrangement of the apparatus.

A Livingston auto-irrigator cone was buried in the sand in each jar. The

auto-irrigator is made of unglazed clay and therefore when filled with nutrient solution readily supplies moisture to the surrounding sand. In the 2-holed rubber stopper in the neck of the cone, a 10-cc. pipette was inserted to serve as a safety reservoir when filling the cone, and also a bent glass tube leading over the edge of the jar, to which the flasks of nutrient solution were connected.

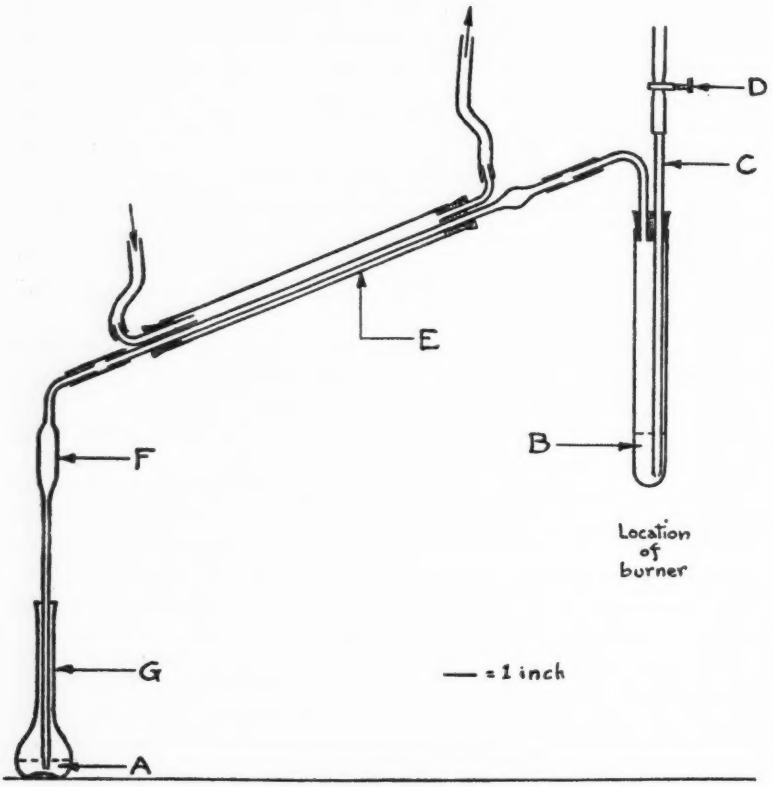

FIG. 1. ARRANGEMENT OF APPARATUS FOR DISTILLATION

A, .1 *N* sulfuric acid; B, digested material; C, tube for holding NaOH; D, clamp on rubber tubing for introduction of NaOH; E, Liebig condenser; F, safety reservoir; G, Volumetric flask.

This arrangement supplies sterile nutrient directly through sterilized containers and tubes, preventing any possible contamination.

By mixing a similar quantity of sand with enough water to bring it to the proper consistency for planting, the amount to be added to each jar was determined. Just before this was added, approximately 5 gm. of powdered calcium

carbonate was mixed with the sand to insure an excess of lime which favors the growth of the root-nodule organisms.

After the addition of the water, two Pyrex glass cylinders, $1\frac{3}{4}$ inches in diameter and 8 inches tall were set upright on the surface of the wet sand, which stood at a level about an inch below the top of the jar. They were held in place by fine, washed gravel which was filled in around the tubes and reservoir pipette. A heavy layer of non-absorbent cotton was placed over the surface of the gravel, which served to keep the cotton from becoming wet through absorbing moisture from the sand. Then the whole jar was tightly

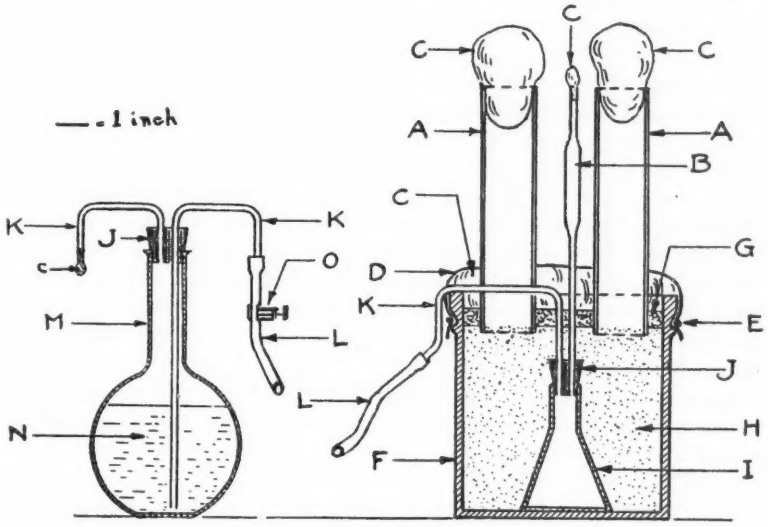

FIG. 2. APPARATUS FOR BACTERIOLOGICALLY CONTROLLED CULTURES

A, Pyrex glass cylinders—plant chambers; B, 10-ml. pipette-reservoir; C, cotton—non-absorbent; D, waterproofed paper; E, string; F, 1-gallon earthenware jar; G, washed gravel; H, glacial sand; I, Livingston Auto-irrigator cone; J, two-holed rubber stopper; K, bent glass tube; L, rubber tubing; M, 1-liter Pyrex flask; N, sterile nutrient solution; O, screw clamp.

covered with heavy waterproof paper, holes being cut in it, which fitted closely around the cylinders and the pipette, and also fitting over the bent tubes from the irrigator at the edge of the jar.

Cotton plugs were then fitted into the large glass cylinders which served as plant chambers and also small plugs closing the top of the pipette and the end of the irrigator supply tube.

At this stage each jar was weighed and numbered so that after sterilization enough sterile nutrient solution could be added to bring it up to its original weight and optimum condition for planting. The jars were then autoclaved at 15 pounds for 18 hours of intermittent sterilization.

The nutrient solution to be used in these sets was made up in 1-liter flasks, 100 cc. of a stock nutrient solution being used to 900 cc. distilled water. The stock nutrient contained 6 gm. of the mixed salts previously described, to 1 liter of distilled water. In each flask was placed a 2-holed rubber stopper fitted with two bent glass tubes, one extending to the bottom of the flask and the other a short bend with cotton plug in the free end. To the longer one was attached about 3 feet of rubber tubing. The end of this tubing was wrapped in paper, held with a paper clip. These flasks were then sterilized in the autoclave at 15 pounds for 15 minutes.

After the sterilized culture jars had cooled, (half a day in the autoclaves and over night outside), they were weighed again and the loss of weight in sterilization was made up by the addition of nutrient solution through the rubber tube on the flask which was connected to the irrigator supply tube on the jar. This brought the sand again to optimum moisture conditions.

Throughout the growth of these controlled sets, the jars were kept at constant weight by the addition of nutrient solution at weekly intervals.

Ten seeds, sterilized in mercuric chloride, were dropped to the surface of the moist sand in each Pyrex cylinder from a flamed platinum loop and the cotton plug immediately replaced. The inoculum was made up in sterile water from two or three tubes of 5-day-old mannite agar slant cultures, half of the bacterial suspension being poured over the seeds in each cylinder. As a final precaution against contamination, the paper covering of the culture was thoroughly sprayed with shellac.

Figures 2 and 3 of plate 1 show the arrangement of the cultures after they had been taken to the greenhouse. The harvesting of the crop and the determination of dry weights and nitrogen content were carried out as previously described.

Sets IX and X were limited to those cultures which had been rather consistently "best" or "poorest," and they were grown at the same time. Planted and inoculated March 30, 1931, reinoculated May 4, they were harvested July 17. Strains I, VI, IX, and 9 were selected as "very good" strains, and V, 6, and 7 as "very poor."

Plate 2 shows contrasting growth in bacteriologically controlled cultures.

Tables 2 to 5 show the comparison in the development of the crops in the different sets, when inoculated with the various strains of *Rhizobium*. Set II, with open-pot cultures, is included, since it shows wide variations from the controlled sets, in many instances. The measurement of tops and roots of the clover plants at harvest time was not begun until the fifth set.

Field Plots

Since the 10 sets of cultures grown on sterilized sand indicated that inoculation with certain strains would, with reasonable consistency, produce a very good crop, whereas others would show only a fair or poor growth, it was decided to try out these strains in the field.

TABLE 2

Summary of appearance of crops

CULTURE NUMBER	SET II*	SET III*	SET IV	SET V	SET VI	SET VII	SET IX	SET X
I	Medium	Medium	Very good	Very good	Very good	Medium	Poor	Poor
II	Medium	Medium	.....	Medium	Very good	Very good	.....	.....
III	Very good	Medium	.....	.....	.....	.....	.....	.....
IV	Very good	Very good	.....	.....	.....	.....	.....	.....
V	Poor	Very good	Poor	Fair	Fair	Fair	Fair	Fair
VI	Poor	Very good	Very good	Very good	Very good	Very good	Medium	Very good
IX	Medium	Medium	Very good	Very good	Very good	Medium	Fair	Very good
1	Medium	Medium	Medium	Medium	.....	Very good	.....	.....
2	Medium	Poor	.....	.....	.....	.....	.....	.....
3	Medium	Medium	Very good	Fair	.....	Very good	.....	.....
4	Poor	Medium	Poor	.....	.....	.....	.....	.....
5	Medium	Poor	.....	.....	.....	.....	.....	.....
6	Medium	Medium	Medium	Medium	Medium	Fair	Very good	Very good
7	Poor	Medium	Medium	Poor	Poor	Fair	Fair	Fair
8	Medium	Very good	Very good	Medium	Fair	Poor	.....	.....
9	Medium	Poor	Very good	Very good	Very good	Medium	Very good	Very good
10	Medium	Medium	Medium	Very good	Medium	Medium	.....	.....
11	Very good	Poor	Poor	.....	.....	.....	.....	.....
12	Medium	Poor	.....	Poor	Poor	.....	.....	.....
13	Medium	Medium	.....	.....	.....	.....	.....	.....
14	Very good	Medium	Very good	Medium	Medium	Fair	.....	.....
15	.....	.....	.....	Poor	Poor	Poor	.....	.....
A	Medium	Poor	Poor	Poor	Poor	Poor	Poor	Poor
B	Medium	Very good	Poor	Poor	Poor	Poor	.....	Poor
C	.....	Poor	.....	Poor	Fair	Poor	.....	.....
D	.....	Poor	.....	.....	Poor	Poor	.....	.....
E	.....	Medium	.....	.....	.....	.....	.....	.....

* Open pot cultures; the others were bacteriologically controlled.

TABLE 3

Measurement and nodulation of crops V, VI, VII, IX, and X

CULTURE NUMBER	SET V			SET VI			SET VII			SET IX			SET X			AVERAGE MEASUREMENTS	
	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots	Nodules*	Tops	Roots
I	6.25	6.25	a–k–u	4	4	u	3.25	8.75	c–m–u	2.75	10	d–s–u	3.5	8	.....	4	7.4
II	5	5	d	4	5	l–s–u	5.25	9	a–l–u	.....	.....	.....	.....	.....	.....	4.8	6.3
V	4.25	4.75	a–l–w–u	4	3	a–l–s–u	3.5	7.5	b–m–s	3	7	b–l–w	2.25	5.5	.....	3.4	5.9
VI	4	6	s–k	4.5	4.5	k–u	4	8	l–u	5	9.5	a–m–s	7	9.5	.....	4.9	7.5
IX	4.25	4.25	k–u	4.5	4	u	3	8	m–s	3.5	7.5	k–s–u	4.5	7.5		4	6
1	4	5	d	.....	.....	.....	4	8.5	a–m–s–u	.....	.....	.....	.....	.....	.....	4	6.8
3	4.5	5.5	d–k–u	.....	.....	.....	4.5	7	a–l–w	.....	.....	.....	.....	.....	.....	4.5	6.3
6	3.5	7	d–s	3.5	5	a–s	3	8	k–s–v	3.5	6.5	k–s–u	4	8	.....	3.5	6.9
7	2	5	b–k–t	1.75	2.75	b–k–s–t	3	7.5	m–v	4	9	a–m–t	3	7	.....	2.8	6.3
8	3.5	6.5	None	1.75	5	none	1.5	4.5	none	.....	.....	.....	.....	.....	.....	2.3	5.3
9	4.5	4.5	a–s	4	4.5	a–l–s–u	3	5.5	d–l–u	4.5	7	u	5.5	8.5	.....	4.3	6
10	6	6	l–s–u	3.5	4.5	a–s	3.5	6.5	l–w–v	.....	.....	.....	.....	.....	.....	4.3	5.7
12	2	6	none	2	4	none	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	5
14	4.5	2.5	d–k–u	4	4	d–l–u	3	9	d–m	.....	.....	.....	.....	.....	.....	3.7	5.2
15	2	5	none	3.5	4.5	d–k–v	1.5	6.5	d	.....	.....	.....	.....	.....	.....	2.3	5.3
A	2.25	7	none	2	3.5	none	1.5	3.5	none	2	10	none	2.5	7.5	none		
B	2.25	8	none	2.25	5	none	1.5	5	none	.....	.....	.....	2.5	7	none	2.14	6.5
C	3.75	6.25	none	2.5	5	none	2	7	none	.....	.....	.....	.....	.....	.....		
D	.....	.....	.....	1.5	7	none	2.25	9.75	none	.....	.....	.....	.....	.....	.....		

* The key to the description of nodulation is as follows: a, abundant; b, very abundant; c, fairly abundant; d, few; k, small; l, large; m, medium; n, all sizes; s, scattered; t, on all roots; u, on upper roots; v, on smaller roots; w, well scattered.

All measurements in length of tops and roots are in inches.

It must be remembered that in the microbiological equilibrium of the soil in nature, *Rhizobium* is only one of many microorganisms living in a given location and, as such, must maintain itself against competition in sufficient numbers and with enough virulence to be effective in nitrogen fixation.

TABLE 4

Total nitrogen in crops

Set No.	II	IV	V	VI	VII	IX	X	
Duration of growth	4 mos.	2½ mos.	3 mos.	3 mos.	4 mos.	3½ mos.	3½ mos.	AVERAGE FOR SETS* V, VI, VII, IX, X
Season	Feb.–May	June–Aug.	Aug.–Nov.	Sept.–Dec.	Jan.–May	Mar.–July	Mar.–July	
Culture number	Total nitrogen in mgm. in entire crop							
I	175.58	2.56	6.13	6.52	3.87	5.00	5.74	5.45
II	173.55	.....	5.44	5.26	5.06	.....	.....	5.25
III	185.48	.....	.....	.....	.....	.....	.....	.....
IV	148.79	.....	.....	.....	.....	.....	.....	.....
V	70.45	1.01	3.67	5.62	3.75	3.93	5.41	4.48
VI	77.38	1.94	5.36	5.80	4.75	6.45	6.64	5.80
IX	190.68	1.40	5.63	7.47	3.46	10.37	7.38	6.86
1	99.28	2.57	4.42	.....	5.78	.....	.....	5.10
2	103.62	.....	.....	.....	.....	.....	.....	.....
3	159.49	1.64	4.22	.....	4.54	.....	.....	4.38
4	185.08	0.58	.....	.....	.....	.....	.....	.....
5	123.47	.....	.....	.....	.....	.....	.....	.....
6	126.16	1.88	6.16	6.76	2.23	5.32	6.26	5.35
7	85.85	1.57	2.32	3.48	2.03	5.20	6.00	3.81
8	103.10	1.51	3.27	3.82	1.97	.....	.....	3.02
9	154.82	1.57	1.15	4.75	4.38	8.04	7.52	6.17
10	76.72	1.15	5.06	5.74	3.73	.....	.....	4.84
11	185.41	0.90	.....	.....	.....	.....	.....	.....
12	159.95	.....	1.46	4.72	.....	.....	.....	3.09
13	145.51	.....	.....	.....	.....	.....	.....	.....
14	232.04	1.87	5.82	5.83	3.84	.....	.....	5.16
15	.....	.....	1.52	2.49	1.32	.....	.....	1.78
A	83.11	0.58	1.57	4.19	0.65	2.75	4.61	2.35
B	158.51	1.06	1.59	3.63	2.19	.....	4.79	3.05
C	.....	.....	1.65	3.72	1.85	.....	.....	2.61
D	.....	.....	.....	2.56	2.79	.....	.....	2.67

* Set II, being an open pot set, was omitted from the average.

Set IV was omitted from the average because its shorter growth period made it incomparable with the others.

Very small experimental plots were prepared in a cherry orchard between the rows of trees, the space having been previously covered with grass. The plots were 1 foot square; they were carefully spaded and prepared, free of larger roots, and were kept weeded. Five grams of seeds, (approximately 100) of *Trifolium pratense* were planted in each plot, the seeds having been first

inoculated by soaking 5 hours in a bacterial suspension made from two tubes of 5-day-old cultures. Six very good cultures and three poorer ones were chosen as the strains to be tested, and three control plots, uninoculated, were planted, the seeds having been first soaked in water. Three months after planting, the following observations were made on the development of the

TABLE 5

Percentage nitrogen in crops

CULTURE NUMBER	SET II	SET IV	SET V	SET VI	SET VII	SET IX	SET X	ALL SMALL CROPS, AVERAGE PER CENT N
I	1.54	1.41	1.51	1.99	0.95	1.07	1.23	1.36
II	1.64	.....	1.33	0.79	1.02	.....	.....	1.05
III	1.24	.....	.....	.....	.....	.....	.....	.....
IV	0.97	.....	.....	.....	.....	.....	.....	.....
V	0.90	0.88	1.16	1.78	0.74	0.80	0.49	0.98
VI	0.98	1.55	1.37	1.37	1.24	1.09	1.39	1.37
IX	1.33	1.53	1.49	1.85	1.20	1.86	1.05	1.47
1	0.67	1.74	1.22	.....	1.11	.....	.....	1.36
2	0.87	.....	.....	.....	.....	.....	.....	.....
3	0.90	0.83	1.47	.....	1.00	.....	.....	1.10
4	1.08	0.75	.....	.....	.....	.....	.....	0.75
5	1.26	.....	.....	.....	.....	.....	.....	.....
6	0.98	1.62	1.60	1.26	0.77	0.86	0.97	1.18
7	1.13	1.71	0.89	0.73	0.80	0.99	0.92	1.00
8	1.04	1.30	0.91	0.58	0.61	.....	.....	0.85
9	1.27	1.51	1.17	1.33	0.81	0.94	0.99	1.13
10	0.54	1.27	1.33	1.57	1.31	.....	.....	1.40
11	1.13	0.88	.....	.....	.....	.....	.....	0.88
12	1.36	.....	0.43	0.84	.....	.....	.....	0.64
13	0.93	.....	.....	.....	.....	.....	.....	.....
14	1.56	1.92	1.33	1.14	0.88	.....	.....	1.32
15	.....	.....	0.46	0.83	0.80	.....	.....	0.70
A	0.69	0.52	0.79	0.64	1.13	0.39	0.34	
B	1.14	0.63	0.48	0.83	1.15	.....	0.50	0.72
C	.....	.....	0.83	1.17	0.81	.....	.....	
D	.....	.....	.....	0.81	0.64	.....	.....	

crop, based on amount of growth, color and size of leaves, and general condition of the plants.

Plate 3 shows some of the field plots in this test.

Agglutination tests[3]

Since the serological differentiation is the most specific means of separating physiologically distinct strains, the next step was to test all strains for their agglutination reactions.

[3] This portion of the work was carried out by Miss Glenna Wurth, under the direction of the author.

Five antisera were obtained from rabbits following injection with five selected strains of bacteria as inocula. Cultures, VI, IX, and 9 were chosen as consistently good strains, and cultures V and VII as poor ones. The sera were tested with their homologous antigens (suspensions of young cultures of the bacteria used for injection) and produced agglutination. When tested against all the other strains, no agglutination occurred.

Normal blood serum from an uninoculated rabbit was tested against each of the five antigens used for inoculation and showed no agglutination.

From these results it can be concluded that with the five sera used, six different agglutination groups are indicated, each serum agglutinating only its homologous antigen, and those not agglutinated constituting a sixth group. The fact that there is no cross-agglutination leads to interesting speculations as to what might have happened had sera for all 14 strains been available and suggests the possibility of demonstrating as many as 14 groups—one for each strain. The results do seem significant, however, in that each strain reveals the serological characteristics ordinarily associated with a distinct species.

TABLE 6

Field test

CULTURE NUMBER	RATING BEFORE TEST	GROWTH IN THE FIELD
I	Very good	Very good
II	Very good	Very good
V	Fair	Medium
VI	Very good	Very good
IX	Very good	Good
3	Very good	Very poor
6	Poor	Poor
7	Poor	Poor
9	Very good	Best
A		Very poor
B		Fair
C		Fairly poor

DISCUSSION OF RESULTS AND CONCLUSIONS

The open-pot method for testing the efficiency of different strains of bacteria was proved unreliable, since *all* cultures, even controls and those inoculated with negative strains, showed the presence of nodules. The comparison of growth in the three sets of open-pot cultures shows very inconsistent results in many cases, even to one check culture in set III producing as fine a crop as any inoculated culture.

The bacteriologically controlled cultures can be relied upon to give results without contamination. In none of the 20 controls grown in connection with the various controlled sets was there a single nodule produced and growth was generally poor. Therefore, the results obtained from inoculation with any given culture can be interpreted as due to that strain alone.

These bacteriologically controlled cultures show rather consistent variations suggesting the existence of physiologically different strains of *Rhizobium trifolii*. Certain strains stand out as having stimulated very good growth throughout most of the controlled cultures,—for example, IX, VI, and 9. Others have been consistently poor, or at best only fair, as V and 7.

A close observation of the growth of these organisms on agar shows cultural differences correlated with the different strains. The "fast growers" and those producing larger quantities of gum seem to be more efficient in their relations to the host plant, whereas the poorer strains produce little gum and a scanty slow growth.

A rather consistent contrast in the nodulation and measurements of roots and tops of the better and poorer strains is apparent. A study of table 3 at this point shows that the more efficient strains have a ratio of from 1:1 to 1:2 in the height of tops to length of roots, whereas the poorer strains and checks have roots that are at least twice as long as the tops. This excessive develop-

TABLE 7

Efficiency in nitrogen fixation

	AVERAGE TOTAL NITROGEN	N
	mgm.	*per cent*
For all check cultures in controlled sets	2.75	0.72
For several negative cultures	1.78–3.02	0.70–0.88
For two notably poor strains	3.81–4.48	0.98–1.0
For the better strains	5.25–6.86	1.13–1.47

ment of the root system seems to be correlated with absence of nodules or infection by an ineffective strain of bacteria.

In nodulation a consistent difference is shown in that the poorer strains have the usually small nodules thoroughly scattered over the entire root system, whereas in the better strains the nodules are larger and distributed chiefly on the upper roots. This variation in distribution of nodules has also been noted by other observers.

The efficiency in nitrogen fixation shows fairly consistent variation, both in the total nitrogen content of the crop and in the percentage of nitrogen. The contrast in efficiency of cultures is brought out in table 7.

The "efficient strains" maintained a high efficiency throughout the sets, and the poorer, less efficient strains remained so. This is contrary to the result described by Allen and Baldwin (1) following a plant passage, though they pointed out that the changes in efficiency were more marked after several passages and in this investigation there was only one between sets IV and V.

The experiments with field plots, although small in number, give a definite suggestion as to the possibilities of inoculation in nature's dynamic habitat, the soil; namely, that inoculation of seed with the various strains yields results corresponding to those obtained under bacteriologically controlled conditions.

The efficient strains were able to maintain themselves against competition and greatly increase the yield of the crop. The poorer strains were unable to compete successfully, and the crops were even somewhat poorer than the uninoculated controls.

This close correlation suggests therefore that the bacteriologically controlled conditions as supplied in sets IV–X constitute an efficient method of testing out the differences in strains, differences which would presumably maintain themselves in inoculation of seed for growth in the field.

The aforementioned set of experiments suggests that efficient strains, such as 1, 9, 14, I, II, VI, IX could be used for inoculation of *Trifolium* with a definite probability of highly increasing nitrogen fixation. Of these very good strains, IX has proved to be the most efficient, increasing the total nitrogen content of the crop 149.4 per cent over the uninoculated controls. Dunham and Baldwin (16) have shown that increased plant growth results from inoculation of seed with an effective strain even when it is sown in soil carrying an ineffective strain. On the other hand, inoculation of seed with an ineffective strain is detrimental to plant growth, even if the seed is planted on soil already carrying an effective strain of the organism. They further state that the results of these studies indicate the necessity of using for seed inoculation only effective strains of the nodule organism, since definite detrimental results may occur from the use of ineffective strains. It is probable that many of the favorable results obtained from the use of inoculated seed on old land are due to the introduction of more effective strains of the organism; also that many of the failures to produce increased plant growth by seed inoculation are due to the use of ineffective strains of the organism.

ECONOMIC BEARING OF THE PROBLEM

That the need of increasing the yield and nitrogen content of leguminous crops in the United States is a real problem was emphasized by Pieters (42) in 1924, when he showed that the culture of red clover in the East is gradually declining. Lipman (40) considered the problem of nitrogen fixation under field conditions so vast and many sided as to assume the proportions of a major factor in our national economy. His suggestion for solution of the problem was that more efficient types of symbiotic legume bacteria, as well as non-symbiotic nitrogen-fixing organisms, must be sought, since commercial nitrogen can supply only a small fraction of the nitrogen requirement of crops.

To this end, commercial cultures of *Rhizobium* are available to agriculturalists for inoculation of seed, but unfortunately, the predicted result has not always followed. Moreover, various commercial preparations have been declared "valueless," "of no practical value," "not satisfactory, and failed to produce nodules" after repeated tests. Among this number have been "Nitro-bacteria soil vaccine" (37), "Soilvita" (18), and "Nitrobion" (38).

It is interesting to note at this point that of the "imported strains" in the foregoing investigation, five of the fifteen were from commercial culture pre-

parations. A study of preceding tables reveals the significant data, given in table 8, regarding these commercial cultures.

When these results are compared with those for uninoculated check cultures in the controlled sets in which the total nitrogen was 2.75 mgm., or 0.72 per cent, and with the nitrogen content of crops inoculated with culture IX, the most efficient strain, showing a fixation of 6.86 mgm., or 1.47 per cent, nitro-

TABLE 8

Summary of data on commercial cultures

CULTURE NUMBER	HISTORY	POSITIVITY	GROWTH	NODULATION	NITROGEN	
					mgm.	*per cent*
7	From Nodogen Culture in 1922, replated and tested 1922–27 and always found positive. A subculture from "Trifolium 205" from Madison, Wis., designated by Wilson, Hopkins, and Fred (55) as a "good nitrogen-fixer"	All but one test + i.e. 4+	Consistently poor to fair	Very abundant small nodules on all roots (controlled sets)	3.81	1.0
11	"A. Dickinson Co. Nod-o-gen Clover" received from Amherst	4 tests−	Principally poor	Very few on upper roots (open pot set)	0.90	0.88
12	"Mulford Co. 1927 Clover"—from Amherst	4 tests−	Poor	None in controlled sets	3.09	0.64
13	"Earp-Thomas Co. Homogerm-Clover" —from Amherst	All tests−	Medium in open pot sets	Few to fairly abundant in open pot sets	....	....
14	"Earp-Thomas Co. Farmogerm Clover" —from Amherst	All tests +	Very good to medium	Few, medium to large, on upper roots (controlled sets)	5.16	1.32

gen, it becomes evident that strains of *Rhizobium are available* which are more efficient than some of those being supplied commercially. Even the two more efficient of these five commercial strains, 7 and 14, show an *increase* in total nitrogen of only 38 per cent and 87 per cent respectively over the controls, whereas IX could be expected to yield 149.4 per cent more nitrogen to the crop than would be present if it were uninoculated.

It would seem, therefore, that a real economic service might be rendered by obtaining *tested* strains for which such quantitative data for crop improvement have been determined under bacteriologically controlled conditions. The fact that certain strains have consistently maintained themselves in field tests also proves this to be a practical and effective method for determination of efficient strains of *Rhizobium trifolii*.

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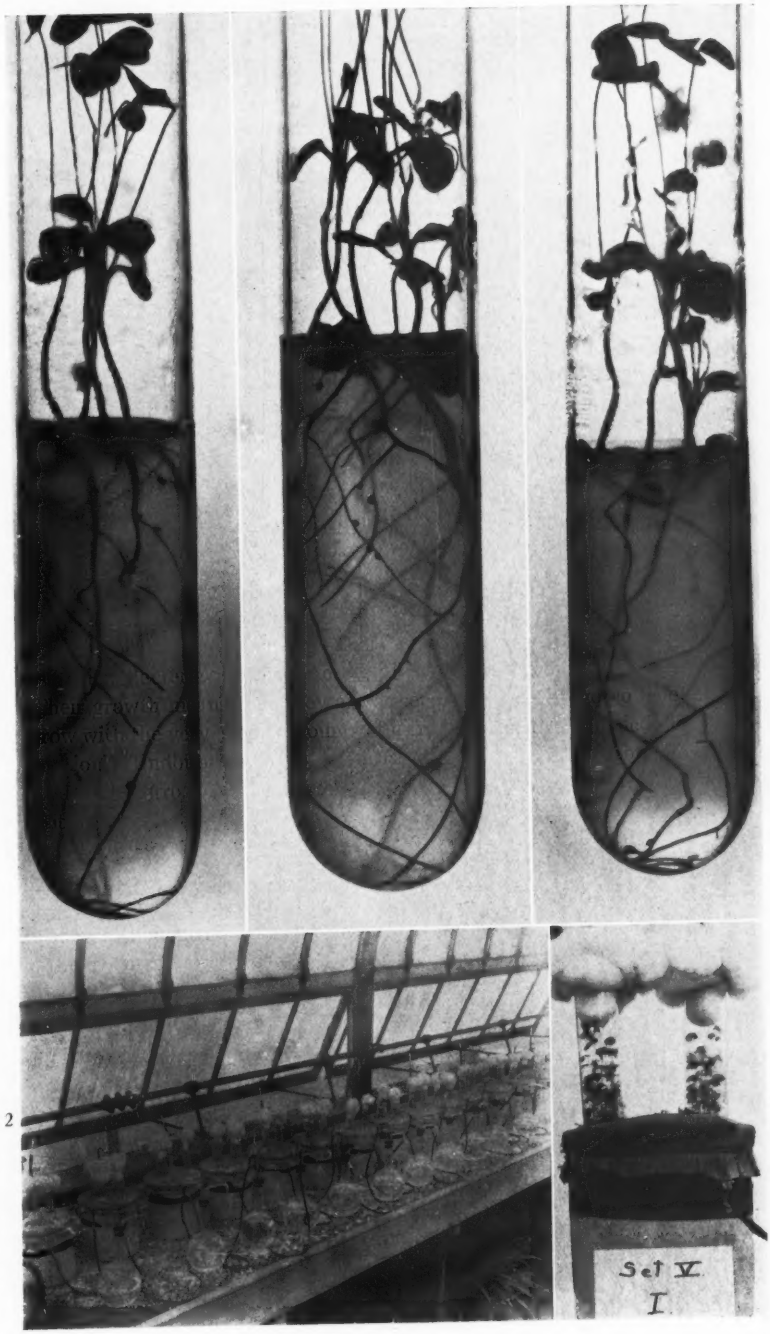
PLATE 1

CULTURES OF RHIZOBIUM TRIFOLII

FIG. 1. Test tube cultures showing nodule production with the cultures IX, I, and VI, all locally isolated strains.

FIG. 2. General set-up of cultures in the greenhouse.

FIG. 3. An individual culture.



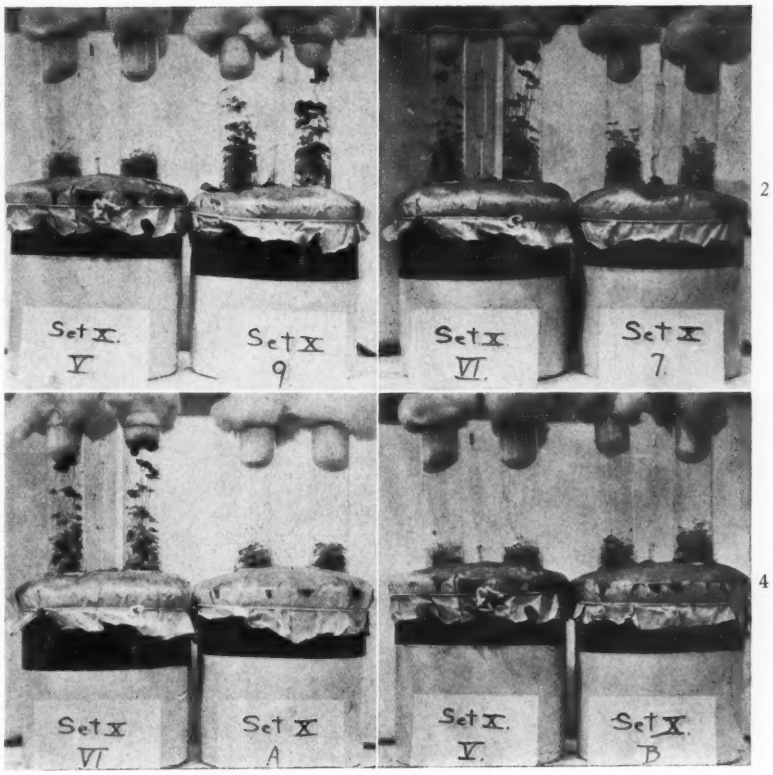
2 3

FIGS. 1–3

PLATE 2

CONTRASTING STRAINS AS TO GROWTH OF CULTURES IN SET X

FIG. 1. Comparison of strains V and 9.
FIG. 2. Comparison of strains VI and 7.
FIG. 3. A very good culture, VI, compared with a check, A.
FIG. 4. A very poor culture, V, compared with a check, B.

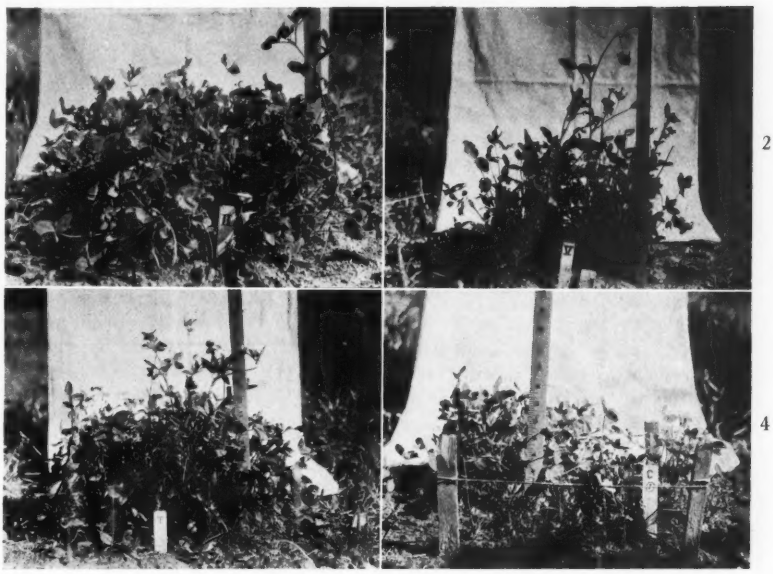


FIGS. 1–4

PLATE 3

FIELD PLOTS OF RED CLOVER

FIG. 1. Plot inoculated with culture II. Measuring stick is in inches.
FIG. 2. Plot inoculated with culture V.
FIG. 3. Plot inoculated with culture I.
FIG. 4. Uninoculated control.



FIGS. 1–4

PRELIMINARY STUDIES IN THE USE OF NITRATE OF SODA ON CERTAIN INDIANA SOILS[1]

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Nitrate of soda has been recognized as one of the standard nitrogen carriers for over a century. Its value as a fertilizer has been tested in comparison with other nitrogenous fertilizers, but definite information on the effects of the time and rate of application upon the yield and quality of the grain is limited.

Nitrate of soda has been commonly used for many years. Kimberly (10), in 1839, wrote: "Saltpeter was known and used as long since as the time of Virgil and we find notice or hint of the effect of nitre or nitrous water worth the attention of farmers in the Sylva of the Bacon, published in the year 1670." He likewise relates various experiments in which the English farmers had tested nitrate of soda down to 1828. The teachings of Liebeg (11) caused considerable opposition to the practice of using nitrate. However, this period of conflict stimulated a more thorough test of the material and by the latter part of the nineteenth century nitrate of soda was accepted as a superior source of nitrogen.

Nitrate fertilization was introduced into the United States about the latter half of the nineteenth century, and as a result various experiments were established to determine its value as a fertilizer. Very favorable increases were obtained by McBryde (12) from its use as a side-dressing for corn and by Hayward (8), as a top-dressing for wheat. These tests were then followed by the work of Voorhees (18), who obtained a substantial increase from a 100-pound application on timothy, and by Atwater and Phelps (1), who obtained net profits of $3.00, $6.60, and $4.47 when they applied nitrate at the rate of 25, 50, and 75 pounds to the acre, respectively.

Patterson (15), Duggar and Williamson (5), Moores and Roberts (13) tested the value of nitrate on corn and they concluded that the highest yields were obtained when the nitrate of soda was applied as a divided application, one-half at planting time and the remaining half at the stage of most active growth.

Bizzell and Morgan (2), Taylor (17), Hutcheson (9), and Harvey (7) reported experiments on the value of nitrate on grass land and concluded that nitrate could be used on each of the respective soils with profit. The smaller rate, as 100 to 200 pounds an acre, seemed to be the best when applied in April.

The fertilizer test conducted in Alabama by Williamson, Appleton, and Helm (19), and by Cauthen and Williamson (3) showed that a moderate rate, from 120 to 200 pounds to the acre, was the most economical, and that the proper time for making the application was either 6 weeks after planting time or when the corn had attained a height of 2½ feet.

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[1] These data are taken from a thesis presented at Purdue University in partial fulfillment of the requirements for the degree of Master of Science. Published with the approval of the director of the Purdue University Agricultural Experiment Station.

[2] Assistant in agronomy, Cornell University. The writer expresses his appreciation to Prof. A. T. Wiancko for his assistance during the course of the investigation; to Prof. G. P. Walker for assistance with field work; and especially to the Chilean Nitrate of Soda Educational Bureau, which made possible the fellowship for conducting this investigation.

This brief summary of the literature shows that nitrate of soda can be used profitably, but still there was some disagreement on the best time and rate of applying the material. Also, the results obtained were applicable only to the section where the tests were conducted, so it seemed desirable that more definite information on the use of nitrate should be obtained by further field experimentation. Thus, in the spring of 1927 this investigation was begun in order to obtain more specific information as to the value of nitrate of soda on certain Indiana soils. The soils chosen for this work included some of the more important soils in Indiana. For the most part, the locations were established on the outlying fields of the Purdue University Agricultural Experiment Station or nearby.

EXPERIMENTATION

Field tests were conducted on corn, wheat, and timothy to determine the influence of nitrate of soda when applied at different times and various rates upon the yield, quality, and protein content of these three crops.

The laboratory studies were made on composite samples taken from the respective plot treatments at harvest time. The $\frac{1}{20}$-acre plot (14 feet by 155.5 feet) was used for all tests.

Corn experiments

The general plan of plot treatment for corn, as given in table 1, provided for a basal application of 300 pounds of 0–12–12 fertilizer to the acre at planting time, either by the broadcast method or in the row with a Planet Jr. fertilizer drill, and the application of the nitrate later at similar stages of growth at each location. The June applications of nitrate were made when the corn was approximately 18 inches high, and the July applications were made when the corn had attained a height of 3.5 to 4 feet. These stages for side-dressing corn were also recommended by Moores and Roberts (13) and by Duggar and Williamson (5). The quality of corn was determined at harvest time upon a basis of maturity and moisture and then was divided into sound and unmarketable classes. The sound corn included all sizes of ears that were sound and solid, whereas the unmarketable corn included ears of all sizes that were soft, moldy, or chaffy.

Table 2 presents the yield obtained from all the fertilizer tests on corn from every location established during 1927 and 1928. Several of the locations included the average of 2 years, whereas the others are the new tests established in 1928. More tests were laid out than are shown in table 2, but since some of these were coöperative tests no data were obtained on account of failure on the part of the coöperators.

The yield data of all plots are on a comparable basis as far as the stands were concerned. The number of stalks on each plot were counted and the actual yields were then corrected to the average stand of the entire series.

The soil of the Wanatah test, which is described as a light colored sandy

TABLE 1

Plan of treatment for corn experiment

PLOT NUMBER	TREATMENT AND RATE PER ACRE
1	300 pounds 0–12–12 broadcast
2	300 pounds 3–12–12 broadcast
3	300 pounds 0–12–12 50 pounds nitrate in July
4	300 pounds 0–12–12 100 pounds nitrate in July
5	300 pounds 0–12–12 broadcast
6	300 pounds 0–12–12, 50 pounds nitrate in June 50 pounds nitrate in July
7	300 pounds 0–12–12, 100 pounds nitrate in June 100 pounds nitrate in July
8	200 pounds 0–12–12 broadcast 100 pounds 0–12–12 in row
9	300 pounds 0–12–12 broadcast
10	200 pounds 0–12–12 broadcast 100 pounds 3–12–12 in row
11	100 pounds 0–12–12 in row
12	100 pounds 3–12–12 in row
13	200 pounds 0–12–12 broadcast, 100 pounds 0–12–12 and 50 pounds nitrate in row + 150 pounds nitrate when corn was 9 to 12 inches high
14	300 pounds 0–12–12 broadcast

TABLE 2

The effect of nitrate of soda on the yield of corn

Bushels per acre

PLOT NUMBER	VINCENNES 1927–28*	BEDFORD 1927–28	WANATAH 1927–28	WORTHINGTON 1927	NORTH VERNON 1928	FARMLAND 1928	CULVER 1928	AVERAGE 1927–28
1	54.6	53.3	30.6	37.2	41.5	34.6	7.4	39.8
2	61.6	60.8	34.2	40.7	43.6	35.5	10.1	44.3
3	63.3	59.4	38.4	38.2	50.3	33.3	17.4	46.1
4	65.8	60.0	37.5	39.5	54.9	29.5	26.5	47.7
5	59.3	54.8	36.4	43.5	43.3	24.5	8.8	42.5
6	70.4	53.6	44.4	40.8	52.6	27.1	20.3	47.8
7	71.8	53.1	45.3	41.4	57.4	33.5	33.4	50.7
8	63.2	52.0	35.6	43.0	39.4	36.7	12.1	42.3
9	62.9	49.2	33.3	43.9	42.3	30.7	5.9	40.4
10	63.5	53.3	39.4	46.5	51.1	26.8	9.8	44.6
11	63.4	55.8	36.4	41.3	53.1	31.3	8.8	49.5
12	60.7	48.1	36.0	42.5	50.8	40.4	10.7	43.4
13	75.1	68.6	35.7	....	....	30.0	33.4	48.6
14	52.2	57.2	26.1	42.3	45.2	22.3	7.3	38.9

* 1927–28 represents the average of the two years.

loam soil and somewhat acid, responded to nitrate fertilization in that all nitrate treated plots gave an increase, but some proved more economical than others. The data show that the divided applications, one-half in June and half in July, gave the best returns, whereas plot 6, with the 100-pound divided application, gave the greatest net profit per acre. The row applications, including approximately 50 pounds of nitrate at planting time, were not very significant, as these treatments gave contradictory results during the 2 years of the experiment. The increases obtained from plot 13 were hardly sufficient to equal the cost of the nitrate and cost of application.

The difference in quality was very noticeable in the 1928 yields. A large type of Reid's Yellow Dent was the variety grown this year, and as a consequence it was caught by the early September frost. The nitrate of soda treated plots had a greater proportion of sound corn than did the plots receiving no nitrate. However, all of the corn had a very high moisture content and the weight per bushel was calculated on a basis of 90 pounds per bushel.

Only the 1928 yields were obtained on the Farmland test, as the 1927 test was discarded because of the very poor stand. The soil of this test is classified as Crosby silt loam, a poorly drained, heavy upland soil. The quality of the corn was very uniform, all coming under the classification of sound corn. The yields of this test are rather small for nitrate application and hardly equal the cost of the fertilizer. The results of plot 12 are rather erratic and cannot be explained, as the other row applications of the small amount of nitrate at planting time did not give any such increase. This in part was attributed to soil variation, as the plot was rather outstanding all through the season.

The average yields of the Bedford test, located on Bedford silt loam in southern Indiana in the limestone area, show that the July applications were the best. Part of these slight increases for nitrate were attributed to the season rather than the nitrogen. The 1927 season was so wet and cool that the planting of the corn was delayed until a late date. The seasonal conditions were also adverse for proper development during the short growing season. Here again, the 50-pound application gave the highest net return, while plot 2, with approximately the equivalent of 60 pounds per acre, was next in profitableness. Since the weather conditions for the 2-year period were contradictory, the nitrate responses were not outstanding.

The Vincennes test was located on a Gibson sandy loam soil in Southwestern Indiana. The data show that all treatments, irrespective of the amount, gave relatively good response to the nitrate fertilization. The only treatment that failed to give a response was the 3–12–12 applied in the row. However, plot 13, with the same treatment plus an additional amount of nitrate when the corn was over 6 inches high showed that plot 12 suffered from a deficiency of nitrogen later in the growing season. The best treatment was that on plot 13, which gave a 24.8-bushel increase. July seemed to be the best time for making the application on this light sandy soil, and the 50-pound rate gave the largest net profit for the amount invested.

The Culver test, located on Plainfield sand, gave very striking increases for the nitrate, but this was expected, as this soil is extremely low in fertility, as shown by the yields of the check plots. The large increases in yields were obtained from the July applications. Both gave about the same increase in proportion to the amount of nitrate applied. This increase of the July application was most effective, as the corn was at sufficient height to assimilate the nitrate in the most economical manner. The divided applications gave good increases, but were not so effective as half the rate when applied in July. The largest bushel increase, 26.3 bushels, was gained from plot 13, but when the net returns were computed this treatment returned about half the net profit produced by the July applications. Nevertheless, a good return was obtained on plot 13 for the amount invested in nitrate of soda.

TABLE 3

Average protein content of corn as influenced by nitrate of soda during 1927–28

PLOT NUMBER	VINCENNES 1927–28	BEDFORD 1927–28	WANATAH 1927–28	NORTH VERNON 1927	FARMLAND 1928	CULVER 1928	AVERAGE 1927–28
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	7.8	7.8	9.3	6.4	9.2	6.1	8.1
2	7.8	7.4	9.4	6.7	9.2	6.3	8.0
3	8.2	7.6	9.7	7.0	8.4	7.4	8.4
4	8.6	8.1	9.8	7.6	8.6	7.1	8.4
5	7.8	7.8	9.3	6.7	8.9	6.7	8.1
6	8.2	7.9	9.5	7.2	9.1	6.5	8.3
7	8.6	8.5	10.0	8.1	9.6	7.5	8.9
8	8.1	8.2	9.5	7.2	8.6	6.3	8.4
9	7.7	8.0	9.4	6.6	9.6	6.2	8.2
10	7.8	8.1	9.7	7.0	9.2	6.3	8.1
11	8.0	7.8	9.2	7.3	10.0	6.9	8.4
12	7.8	7.6	9.3	7.3	10.2	6.9	8.3
13	8.1	7.6	8.7	...	10.6	7.3	8.6
14	8.0	8.3	9.3	7.7	10.0	6.6	8.5

The Clermont silt loam soil of the North Vernon test is a flat, poorly drained, whitish gray soil of rather low fertility, but is quite responsive to fertilizers when properly drained. The practice of side-dressing corn with nitrate proved very effective, as substantial increases in yields were obtained from all nitrate plots. The outstanding one was the 50-pound application in July which gave a 7.9-bushel increase. The other side-dressings of nitrate gave responses ranging from 10 to 16 bushels, but when compared in proportion to the amount of nitrate applied the 50-pound application gave the most economical returns.

The protein content of corn is summarized in table 3. One readily sees that the protein content is influenced by the soil type and local climatic conditions. The poor soil types as the Clermont silt loam or the Plainfield sand have a much lower percentage than the other soil series of the experiment. However, these local factors have not offset the influence of the nitrate. The data clearly

show that the heavy applications have increased the protein content more than the light rates. The 200-pound application in April gave an increase of approximately 0.8 per cent, whereas the 100-pound application gave an appreciably lower increase. Also, the July application had more effect than the early June application. In general, nitrate fertilization does exert some influence upon the protein content.

Summary of corn experiments. The results of the first 2 years of this experiment show that nitrate of soda can be used as a fertilizer on Indiana soils with a fair degree of profit. The 50-pound application applied in July gave an average increase of 5 bushels of corn an acre, and the 100-pound July application gave an average increase of 5.8 bushels. The 100-pound divided application yielded approximately the same, 5.9 bushels. The 200-pound application gave a 9.3 bushel increase.

The small amounts applied in the row at planting time did not prove practical, as this early stimulation caused the formation of too large a plant. This later caused the plant to suffer because of the lack of available nitrogen.

Wheat experiments

A series of 10 one-twentieth acre plots were used for the wheat experiments that were laid out on bulk wheat fields on various soils of the state. Special care was taken in the choice of these fields for the experiment so that no nitrate fertilizers had been applied on them except for a small amount at seeding time in the fall. The general type of treatment is as follows:

1. No nitrate
2. 50 pounds in April
3. 100 pounds in April
4. 200 pounds in April
5. No nitrate
6. 100 pounds in May
7. 50 pounds in April and 50 pounds in May
8. 100 pounds in April and 100 pounds in May
9. 150 pounds in April
10. No nitrate

The nitrate applications were made on wheat at similar growth stages. The April applications were made when the wheat was approximately 4 inches high, and the May applications were made when the wheat was 12 to 15 inches high.

The influence of nitrate of soda on wheat yields is shown in table 4, which includes all locations harvested during 1927 and 1928. The results show that the individual soils responded somewhat differently to the added nitrate, but on the whole the April applications were generally the best. Each test will be discussed separately.

The Vincennes test was located on Gibson silt loam soil. Increases in yields resulted for every treatment. However, the outstanding treatment both

years of the test was the 50-pound application in April, giving a 2.8-bushel increase, while the 100-pound application gave almost as good returns in proportion to the amount of nitrate, with a 5.0-bushel increase. The May applications seemed to be rather late for stimulating the yields in this southern section.

The average yields for the 2-year period on Bedford silt loam did not show very high increases. This in part may be attributed to the 1928 yields, as the stand for this year was thin as a result of severe winter injury. Nevertheless, the early nitrate application encouraged a thickening of the stand. The April treatment was thus sufficient to produce a higher yield than the May application. This test credits the 100-pound April treatment as the best for this soil with an average increase of 4.5 bushels.

TABLE 4

Average results of top-dressing wheat with nitrate of soda in 1927–28

Bushels per acre

PLOT NUMBER	TREATMENT IN POUNDS NITRATE OF SODA PER ACRE	VINCENNES 1927–28	BEDFORD 1927–28	NORTH VERNON 1927–28	LAFAYETTE 1927–28	WANATAH 1927–28	CULVER 1927–28	CONNERSVILLE 1928	AVERAGE BUSHELS 1927–28
1	No nitrate	13.4	16.9	8.5	24.8	13.4	11.8	22.3	14.8
2	50 in April	16.7	17.0	10.3	27.6	21.5	14.6	24.7	17.2
3	100 in April	19.4	17.3	11.0	29.6	24.8	14.6	27.3	19.3
4	200 in April	22.1	21.3	13.2	29.9	28.5	21.3	25.3	23.1
5	No nitrate	15.5	13.3	9.8	24.1	19.1	10.2	19.3	13.6
6	100 in May	....	15.0	11.4	27.0	23.8	....	22.7	18.1
7	50 in April + 50 in May	18.3	16.1	14.0	30.0	23.5	....	31.3	19.9
8	100 in April + 100 in May	23.4	17.9	17.9	31.6	27.6	....	24.7	21.6
9*	150 in April	....	8.0	13.0	22.2	35.0	20.7	26.7	21.1
10	No nitrate	16.3	12.3	9.3	25.0	16.6	12.2	23.7	15.4

* 1928 yields only.

The Clermont silt loam, the soil of the North Vernon test, was responsive to nitrate during the first 2 years of the investigation. This soil is poorly drained and as a consequence is rather late in warming up in the spring, so the natural nitrification is impeded during these early spring months. Thus, nitrate additions were very beneficial both in April and May. The 100-pound divided application, one-half in April and one-half in May, was the outstanding treatment of the test giving a 4.5-bushel increase. This was followed by plot 8 with a similar type of treatment except that a total of 200 pounds was applied. This resulted in an average increase of 6.5 bushels. The 100-pound application in May, 1928, was almost as profitable as the 100-pound divided application, but the average for the 2 years was materially reduced by the lateness of the 1927 application and which was applied close to heading time. As a

consequence, this was too late for stimulating yields. The April treatments were probably not all utilized by the small plants and in all probability some nitrate was lost in the drainage water.

The Lafayette test was laid out in 1927 on Brookston silt loam, a dark colored soil; the 1928 test was laid out on Crosby silt loam which was somewhat lighter in color and contained slightly less native fertility than the Brookston soil. Nevertheless, good increases were obtained for all nitrate treatments during both years. The increases on the Brookston silt loam were not as great as on Crosby, especially the 200-pound April application, which was too heavy for this soil and resulted in a poorer quality of wheat, as a result of lodging of the grain before maturity. Substantial increases were obtained from all the other treatments. The 50-pound application in April was the best in this test, giving a 3-bushel increase; the other nitrate applications were slightly lower in proportion to the amount of nitrate applied.

The response to nitrate of soda fertilization on Plainfield sand at the Culver location was very pronounced for all the different applications. This was expected on this soil, because of its low native fertility, being especially lacking in nitrogen. Since the May applications of this test were spoiled in 1928, no average yields were obtained. However, the 1927 yields showed that additional nitrate applied on this open, sandy soil was beneficial, giving a much greater response when applied either as a divided application or all in May. These data showed that all rates and all times of application gave good returns on the investment. The 50-pound application in April was exceptionally effective, yielding a 2.2-bushel increase over the untreated plot.

The Connersville test was established on Russell silt loam in 1928. The yields for this season gave the 100-pound divided application the advantage, resulting in an increase of 10.2 bushels, whereas 50- and 100-pound applications in April were very good but were slightly lower in their net increases for the amount of nitrate applied. However, these data show that nitrate of soda can be used very effectively up to 100 pounds an acre.

The quality of the wheat was determined on the laboratory studies based on protein content and test weight per bushel. The test weight was measured by the official method, and the official Kjeldahl method was used for the protein determinations. The results for these studies, as given in tables 5 and 6, are based only on the 1928 yields. These data are somewhat variable, being slightly influenced by the local climatic conditions and the soil type. However, the nitrate of soda additions have caused some outstanding differences, mainly, that the April applications gave a lower percentage of protein than the corresponding May applications. There was a direct proportional increase for the April additions in favor of the increased amount of nitrate. The same is true with respect to rates of the May applications. However, the May applications are the highest as they were applied closest to the time of maturity. These results corroborated the studies of Davidson (4), Gericke (6), Shaw (16), and Neidig and Snyder (14) on the protein content of grain.

The test weight per bushel shows slight fluctuations with respect to the individual soil series. On the average, the April applications gave a slight decrease, whereas the May applications gave an increase. In general, the test weight per bushel was increased in cases where there was an abundance of the

TABLE 5

The effect of various applications of nitrate of soda on the test weight per bushel of wheat grain in 1928

PLOT NUMBER	TREATMENT IN POUNDS NITRATE OF SODA PER ACRE	VINCENNES	BEDFORD	NORTH VERNON	CONNERSVILLE	LAFAYETTE	CULVER	WANATAH	AVERAGE
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	No nitrate	59.3	55.4	58.9	57.0	60.2	57.9	57.4	58.0
2	50 in April	59.0	54.7	58.5	57.0	60.1	58.7	57.4	57.9
3	100 in April	58.4	54.7	58.9	57.0	59.8	58.4	57.4	57.8
4	200 in April	57.7	55.7	58.7	57.0	59.8	59.4	58.5	58.1
5	No nitrate	57.5	55.9	59.0	55.0	60.3	58.1	57.5	57.6
6	100 in May	....	54.2	58.0	56.0	61.1	....	58.5	57.7
7	50 in April + 50 in May	57.6	54.6	58.6	56.5	60.7	....	58.2	57.7
8	100 in April + 100 in May	57.6	54.1	55.6	55.0	61.1	....	58.0	57.0
9	150 in April	....	55.0	56.0	56.0	61.4	58.0	58.7	57.5
10	No nitrate	59.0	55.3	56.0	56.0	60.4	58.0	57.8	57.5

TABLE 6

Percentage of protein in wheat as influenced by various applications of nitrate of soda in 1928

PLOT NUMBER	TREATMENT IN POUNDS NITRATE OF SODA PER ACRE	VINCENNES	BEDFORD	NORTH VERNON	CONNERSVILLE	LAFAYETTE	CULVER	WANATAH	AVERAGE
1	No nitrate	9.7	9.0	9.1	9.3	9.1	9.9	9.6	9.4
2	50 in April	10.5	10.5	9.0	9.8	9.3	9.5	9.6	9.7
3	100 in April	10.6	10.7	9.0	9.4	9.5	9.6	9.6	9.8
4	200 in April	11.2	10.5	9.3	9.5	9.6	11.0	10.6	10.2
5	No nitrate	9.6	9.6	9.4	9.5	9.2	10.0	9.4	9.5
6	100 in May	....	11.2	9.0	9.5	10.8	....	10.4	10.2
7	50 in April + 50 in May	11.1	10.9	9.0	9.6	9.4	....	10.1	10.0
8	100 in April + 100 in May	11.2	11.2	9.3	9.6	10.5	....	10.8	10.4
9	150 in April	....	11.1	9.2	9.4	10.5	9.5	10.7	10.1
10	No nitrate	9.7	10.0	8.9	9.4	9.7	9.9	9.1	9.5

other fertilizing elements present, and the reverse condition resulted where there was a shortage of essential elements.

In considering the average return from treating these Indiana soils with nitrate, it is evident that the April top-dressings were the most profitable, especially the smaller rate of application. The 50-pound application gave an

average increase of 2.7 bushels, whereas the 100-pound application gave a 5.0-bushel increase, which was slightly lower in proportion to the amount of nitrate. The 100-pound application, in which 50 pounds was applied in April and 50 pounds in May, gave the largest acre increase, 5.5 bushels. However, when the extra cost of applying the material in two installments is considered, the net profit would be reduced somewhat, and in all probability the single April application would be the most practical for average farm conditions. The 200-pound rate in April gave 7.8 bushels increase, whereas the 200-pound divided application on plot 8 gave only 6.7 bushels.

Timothy experiments

The field tests for timothy were conducted on $\frac{1}{4}\frac{1}{0}$-acre plots but were laid out in duplicate at each location in order to facilitate weighing at harvest time. Thus, all yields recorded are an average of duplicate tests. The general plan for the nitrate experiment is given in the following:

Plan of timothy experiment

1. No fertilizer
2. 100 pounds 0–12–12
3. 100 pounds 0–12–12 plus 100 pounds of nitrate in April
4. No fertilizer
5. 100 pounds 0–12–12 plus 200 pounds of nitrate
6. 100 pounds 0–12–12 plus 300 pounds of nitrate
7. No fertilizer

The average results of applying nitrate of soda to timothy are summarized in table 7 for all locations harvested during 1927 and 1928. The top-dressings were made early in the spring usually when the timothy had made a fair growth and was 4 to 6 inches high.

The North Vernon test was located on Clermont silt loam on adjacent farms for the 2-year period. The meadow both years was a 2-year-old one. The timothy was allowed to dry thoroughly before the weighings were made. The yield data show that all rates of nitrate applied were effective in that all yields were in proportion to the amount of the application. However, the 200-pound rate was the most effective, giving an increase of 1,565 pounds of hay over the check plot, whereas the 300-pound application gave an increase of 2,227 pounds of hay. The differences in maturity were very noticeable, as the crop receiving the heavy nitrate application was slightly earlier, whereas that receiving the lighter application and that from untreated plots were decidedly later. However, the heavy nitrate plots had a tendency to produce a slightly coarser hay.

The Columbus test was laid out on Clermont silt loam in Bartholomew County. This was a 2-year-old meadow. Here again, the response to nitrate was restricted, because of the extremely low fertility of this "slash land" soil. The 100-pound application was the best, producing a 1,064-pound increase,

whereas only 1,343 and 1,930 pounds of hay were the increases received from the 200-pound and 300-pound applications respectively. The effects of the nitrate were shown by a thickening of the stand, restriction of the weeds, and a reduction of clover.

The Monticello test was conducted in White County on light, sandy soil. The beneficial effects of the nitrate were observed soon after the initial application, and at harvest time the nitrated plots were 10 inches taller than the untreated plots. The actual increases for the 2-year period are proportional to the increasing rate of application. However, the 200-pound application was the most economical, giving a 2,173-pound increase, whereas the 300-pound application yielded a 2,424-pound increase. Here again, the 300-pound application was too heavy. This was shown by the coarse woody condition of the stems and a browning of the leaves.

TABLE 7

Average yields of timothy top-dressed with nitrate of soda at each location

PLOT NUMBER	TREATMENT PER ACRE	NORTH VERNON 1927–28	MONTICELLO 1927–28	COLUMBUS 1927	WORTHINGTON 1927	CULVER 1927	CRAWFORDSVILLE 1928	AVERAGE FOR ALL TESTS 1927–28
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	No fertilizer	2,060	2,035	1,040	2,200	1,480	1,210	1,820
2	100 pounds 0–12–12	2,350	2,040	1,360	3,040	1,640	1,210	2,031
3	100 pounds 0–12–12 100 pounds nitrate	3,230	3,080	2,540	4,520	2,420	2,178	2,992
4	No fertilizer	2,205	2,280	1,380	3,260	1,280	1,210	2,015
5	100 pounds 0–12–12 200 pounds nitrate	3,980	4,310	3,000	6,360	3,640	2,672	3,985
6	100 pounds 0–12–12 300 pounds nitrate	4,420	4,560	3,660	6,240	4,560	3,267	4,336
7	No fertilizer	1,975	2,280	1,600	3,900	1,490	1,090	1,994

The Worthing test was established in 1927 in Greene county on a heavy silt loam. This was the first year's crop for this meadow. All through the season the nitrate plots were superior and the increases obtained from this test show that nitrate can be used with profit up to 200 pounds an acre. When nitrate was applied much above this rate, the quality was reduced and the timothy lodged rather badly. The 100-pound application produced a 1,134-pound increase, and the 200-pound application gave an increase of 2,380 pounds.

The Culver test was laid out on sandy loam soil in Marshall County on a new timothy meadow. The nitrate of soda was very effective on this field, as increased yields were obtained for each additional 100 pounds. The 300-pound application gave the largest increase in proportion to the amount of nitrate applied. However, good increases were received from the other nitrate applications. The 100-pound application gave an increase of 846 pounds of hay, and the 200-pound application of nitrate produced a 2,060-pound increase.

The Crawfordsville test was located on Miami silt loam on a 2-year-old meadow. Nitrate of soda was effective in producing increases, but the increases were not so outstanding as on the other soil types used in this experiment. The 100-pound rate was most effective in proportion to the amount applied, giving an increase of 968 pounds an acre. The higher rates of application gave very little for the additional nitrate.

Summary of timothy experiments. The data obtained from the experiments in this project show that nitrate of soda gave increases in hay yields of 927, 1,876, and 2,210 pounds for the 100, 200, and 300-pound applications respectively. Thus, for average Indiana conditions the 200-pound application proved to be the most profitable, giving the best proportionate increase as well as the best quality of hay.

The results show that nitrate of soda applied to timothy meadows in early spring was responsible for stimulating growth, increasing the stand, and restricting the growth of weeds.

SUMMARY

The favorable results obtained from the use of nitrate of soda during the 2 years of this investigation may be in part attributed to seasonal influence. In both years cool weather was prevalent during the early part of the growing season, and caused a general nitrate deficiency. Thus, the nitrate of soda was very beneficial in increasing yields. However, these results may be altered by the local variation in temperature or rainfall.

The average crop increases produced by the nitrate of soda applications on corn, during the 2 years, were barely sufficient to pay the cost of the nitrate. The 50-pound application in July was the only application to give a net profit.

In general, the small amounts of nitrate applied in the row at planting time on corn gave a stimulating effect at first, but caused the crop to suffer from lack of available nitrogen later in the season, thus causing a decrease in yield.

The average of the 2 years' data on applying nitrate of soda to wheat shows that the smaller rates of application, 50 or 100 pounds, were the most profitable, and that the April applications proved to be the best.

The results obtained from top-dressing timothy meadows with nitrate of soda showed that moderate amounts were beneficial in increasing yields, in stimulating growth, and in limiting the growth of weeds.

The protein analyses of the three field crops for the 2 years show that the percentage of protein is raised to a slightly higher degree by late application as well as by a heavier application of nitrate of soda.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: X. EXCHANGE NEUTRALITY AND COMBINING CAPACITY[1]

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The soil colloidal complex represents salts of weak acids (silicic, humic, etc.) and bases (chiefly of aluminum and iron). Because of their weakness these acids and bases neutralize each other only partially, leaving an acid and a basic residue too weak for a mutual interaction. These compounds react therefore amphoterically at higher pH with bases, and at low pH with acids. Their isoelectric point, that is, the point at which the acid and basic residues are equally ionized, depends upon the relative strength of the acid and basic radicals. The soil complex is, with few exceptions, isoelectric on the acid side of neutrality. The acid residue is therefore usually stronger than the basic.

Since the weak acids and bases are mostly themselves highly insoluble compounds it is clear that the composition of the complex can vary within the widest limits. Thus we may have compounds of the following types in which A and B represent trivalent acid and basic groups respectively:

(*A*)	$H_2A\diagdown$ $\quad B\,OH$ $H_2A\diagup$	cation combining power high; anion combining power low; isoelectric pH low; ultimate pH low.
(*B*)	$H_2A - B(OH)_2$	cation and anion combining power intermediate; isoelectric pH intermediate; ultimate pH intermediate.
(*C*)	$\quad\diagup B(OH)_2$ HA $\quad\diagdown B(OH)_2$	cation combining power low; anion combining power high; isoelectric pH high; ultimate pH high.

These formulas are merely given to represent different steps in the ratio between the acid and basic radicals. It is obvious that in a colloidal aggregate containing millions of molecules any composition is possible. We thus have every transition in the composition of soil colloids from those which are high in silica to those having a high sesquioxide content.

Hand in hand with this gradation in composition we find a gradation in the

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amphoteric behavior and reactivity of the colloids as indicated by the foregoing scheme. The power to adsorb and exchange cations, which increases with increasing pH, is proportional to the strength (dissociation) and quantity (equivalence) of the acid residue whereas the power to adsorb and exchange anions, which increases with decreasing pH, is proportional to the strength and quantity of the basic residue. The pH of exchange neutrality (3, VI), that is, the point at which the free ampholytoid combines with an equal number of the cations and anions in a particular salt solution in exchange for H and OH ions, is therefore, like the isoelectric point, dependent upon the composition ratio or, more correctly, upon the ratio of the activity of the acid residue to that of the basic residue.

Since most of the properties of soil colloids are directly or indirectly functions of this dual character to react with acids and bases and with anions and cations it is obviously of the utmost importance to find a simple procedure whereby their reaction capacity and their point of exchange neutrality may be determined. This may be found quite simply by determining the neutralization curve for the electrodialyzed soil in a series of neutral salt solutions to which free acid on the one side and free base on the other have been added as previously described (3, VI). In the following pages we shall present the results of this method applied to various soils and other materials. For a theoretical discussion of the neutralization of amphoteric colloids the reader is referred to the preceding section of this series (3, IX).

In determining the power of a colloid to bind acid or base it is, of course, as imperative that the colloid be in the free acid-base condition as it is that an ordinary acid or base be in the free, uncombined condition when such a determination is to be made. This is most easily accomplished in the case of soils by elctrodialysis. The soil complex is thereby converted into the free acid-base-ampholytoid.

The pH of the electrodialyzed soil represents its ultimate value and expresses the relative strength of the acid and basic residue. The ultimate pH is low in soils whose complex is high in silicic and humic acids, whereas the ultimate pH is high in laterites and other soils high in sesquioxides.

When an electrodialyzed soil is added to a neutral salt solution an exchange acidity is usually developed, since most soils possess a stronger acid than basic residue. (Aluminum hydroxide and some extreme soil types isoelectric above pH 7.0 yield an exchange alkalinity.) The pH developed by the exchange reaction between the electrodialysed soil and the neutral salt solution depends (*a*) upon the amphoteric nature of the colloidal complex of the soil and (*b*) upon the nature of the ions of the salt. Since the neutral salt solution possesses practically no buffer capacity the pH at equilibrium with the electrodialyzed soil will very nearly represent the point of exchange neutrality, that is, the pH at which the anions and cations of the salt are adsorbed in equal numbers. The SO_4 ions displace the OH ions more energetically than do the Cl ions (3, III). This we ascribe to a higher association, i.e., a lower dissociation, of the SO_4 ions.

The pH of exchange neutrality is therefore higher in a Na_2SO_4 solution than in a NaCl solution. The Ca ions displace the H ions more strongly than do the Na ions. The pH of exchange neutrality is therefore lower in $CaCl_2$ than in NaCl (3, V). The point of exchange neutrality, therefore, does not necessarily coincide with the isoelectric point. The former point represents the pH at which the colloid *combines* with an equal number of anions and cations of the salt, whereas the latter point is defined as the pH at which the colloid *dissociates* an equal number of anions and cations.

In the following experiments a normal solution of Na_2SO_4 was employed. The advantage of using a neutral salt solution in determining the neutralization curve of a soil is twofold. In the first place it yields a significant point of origin, or zero point, as represented by the pH of exchange neutrality. In the second place the presence of a large amount of neutral salt greatly hastens the displacement, which otherwise proceeds very slowly upon the additions of small increments of acid or base. The equilibrium position is, however, displaced by the presence of the salt, which causes more of the anions and cations to combine at a given pH.

EXPERIMENTAL PROCEDURE

A series of stock solutions of *N* Na_2SO_4 were prepared by adding increasing quantities of H_2SO_4 on the one side and NaOH on the other. The dotted S curves in the figures marked "Na_2SO_4 solution" show the relationship between the pH (ordinates) and the milliequivalents of acid or base contained in 25 cc. of solution (abscissa).

One gram (except where otherwise stated) of the electro-dialyzed soils or other materials was shaken with 25 cc. of the various solutions and let stand over night. The pH was then determined colorimetrically in the clear supernatant liquid and a curve plotted for each material to the same scale as the curve representing the original salt solutions.

Every material which reacts amphoterically will yield a curve which intersects the salt solution curve. The point of intersection marks the pH of exchange neutrality, whereas the horizontal distances between the two curves yield, at any one pH when projected on the abscissas, the *net* combining power in milliequivalents per gram for acids (to the left) and bases (to the right).

THE REACTION OF DIFFERENT SOIL TYPES

We shall first present the results obtained from three soils which represent extreme variations in composition; namely, the Sharkey, the Sassafras, and the Nipe soils. The colloids of these soils have already been discussed in respect to many of their properties. The ratio of silica to sesquioxide and the cation exchange capacity at pH 7.0 of these colloids will serve to account for the reaction of the soils although the colloids were extracted from different samples. The figures are as follows:

COLLOID	SHARKEY	SASSAFRAS	NIPE
SiO_2 / R_2O_3	3.18	1.89	0.31
Exchange capacity, milliequiv/gram	0.80	0.33	0.04

Figure 28 shows the pH of exchange neutrality in Na_2SO_4 of the three (whole) soils as well as their reaction capacity, or more specifically, their power to bind H_2SO_4 and NaOH in the presence of *N* Na_2SO_4.

The *pH of exchange neutrality* in the Na_2SO_4 solution, i.e., the points of intersection, are found to be: Sharkey, 3.7; Sassafras, 4.7; Nipe, 6.1.

These values for the Sharkey and the Sassafras soils are somewhat higher than those previously reported for the exchange neutrality of their colloids in NaCl (3, VI). This is partly because the SO_4 ions are more strongly adsorbed than the Cl ions. The adsorption of the SO_4 ions will therefore balance the cation adsorption at a higher pH. A different salt effect on the indicator is another factor.

The *ultimate pH*, that is, the pH of the electrodialyzed soils, was determined in a ratio of soil to water of 1:2 and was as follows: Sharkey, 3.3; Sassafras, 4.45; Nipe, 6.0.

The ultimate pH at the dilution investigated was therefore somewhat lower than the pH of exchange neutrality in *N* Na_2SO_4 solution (dilution 1:25). This indicates a strong adsorption of the SO_4 ion but the, here one-sided, salt effect renders a comparison uncertain. These two points are, as already pointed out (3, VI), related to, but not identical with, *the isoelectric point.* All three of these points are governed by the relative strength of the acid and basic residues. The stronger the acid and the weaker therefore the basic residue, the lower will be the pH of either of these points or vice versa. These points might be called *the amphoteric points* of the colloidal complex. (The ultimate pH is a value which is not alone confined to amphoteric colloids, for it is obviously also associated with colloids which are strictly acidic or basic in behavior.)

The amphoteric points are therefore intensity factors and express the quality of the soil complex as distinguished from the quantitative factors and measurements which have in the past chiefly attracted the attention of soil chemists. How useful and how essential a study of these qualitative characteristics of a soil are for an understanding of the soil processes will be brought out later. We shall now turn our attention to the quantitative aspects of the soils as shown in figure 28.

The *cation exchange capacities* at pH 7.0 as determined by leaching the soils with *N* barium acetate, washing and displacing the combined Ba with NH_4Cl, were, in milliequivalents per gram, as follows: Sharkey, 0.41; Sassafras, 0.06 Nipe, 0.04.

If we now compare these values with the quantities of NaOH with which the soils have combined at a pH of 7.0 as shown in figure 28 by the points at which

the neutralization curves intersect the neutrality line, we note an almost perfect agreement in the case of the Nipe and Sassafras soils whereas the Sharkey shows a somewhat lower combining power, or about 0.35 m.e. NaOH as compared to 0.41 m.e. exchangeable Ba. Now it must be remembered that both the nature of the cations and the conditions of the experiments were different in the two cases. The two series of values should therefore not be expected to agree except in a general way. One method is as justifiable as the other for a determination of the "base exchange capacity" of a soil. Neither method corresponds to the equilibrium conditions in the field.

THE "TOTAL" COMBINING CAPACITY

The neutralization curves of the electrodialyzed soils have not been carried beyond a certain point on the alkaline and acid sides because it is quite obvious that such a procedure would not yield the combining power of the soil complex as it originally existed. The soil complex is very unstable and undergoes an extensive hydrolysis and dissolution at high and low pH, culminating in silicates and aluminates on the one side and salts of aluminum and iron on the other.

The "total" combining power or exchange capacity of the colloidal complex is therefore a meaningless term. Furthermore certain soil complexes contain acid radicals too weak to come into play within the ordinary range of soil reactions which at higher pH bind large quantities of bases. Thus laterites having a high content of the weakly acidic, amphoteric alumina possess an extremely low exchange capacity at pH 7.0 whereas at high pH these soils may exceed, in their base binding power, other soils which possess a very much higher exchange capacity at the neutral point. The exchange capacity at pH 7.0 bears therefore no simple relationship to any "total" combining power. The degree of saturation based on any such total might be very misleading.

THE COLLOID CONTENT

The quantity of base adsorbed by a soil at any pH depends upon the combining capacity of the colloid and upon the quantity of the colloid in the soil. The combining capacity of the colloidal complex for bases depends upon the number of equivalents in the acid residue of sufficient strength to become neutralized at the pH in question. For a given soil the quantity of base adsorbed at any pH is proportional to the percentage of colloid present. The point of exchange neutrality, which is a qualitative expression, is independent of the amount of colloid present, obviously within certain limits of concentration. This is expressed in figure 29, which shows the neutralization curves of the Sassafras soil and colloid.

From the quantities of base adsorbed by the two materials at pH 7.0 (or any other pH above the point of exchange neutrality) we find that the colloidal fraction makes up about 31 per cent of the soil. The fact that the point of exchange neutrality is unaffected by the change in the colloid concentration is extremely important, since this constancy permits a characterization of the

soil complex without reference to its quantity, which is practically impossible to determine directly, i.e., by extraction.

EXCHANGE EQUIVALENTS

Figure 30 gives the neutralization curves of the three soils when taken in the proportion of their base combining equivalents at pH 7.0. The proportions were calculated from figure 28, which gives the combining power per gram soil under the same conditions. Eight grams of the Nipe and five of the Sassafras soil should be approximately equivalent to one gram of the Sharkey soil in respect to their power to neutralize NaOH at pH 7.0. It must be emphasized that these weights are equivalent only at the neutral point for which they are calculated.

It will be noted that the curves all intersect at a pH slightly above 7.0. The exactness of this point is of little significance for our present study. The deviation may be due to errors in the interpolation, or it may be a concentration effect. It may also be due to CO_2 which would give the solution a certain buffer capacity. This would make the combining power of a soil appear greater than its true value and would affect soils of low combining power, such as the Nipe, much more than soils of the Sharkey type. If so, more than eight parts of the Nipe should have been taken.

The significant thing about the curves in figure 30 is their form as well as their intersection. The pH of exchange neutrality, i.e., the points of intersection with the Na_2SO_4 solution curve, being unaffected, the slope of the Sassafras and especially that of the Nipe soil is greatly flattened. The buffer capacity per soil equivalent (not per gram soil) is greatest by far in the case of the Nipe soil, which adsorbs the base within the pH range of about 6.1 to 7.2 whereas the Sassafras covers a range from 4.6 to 7.2 and the Sharkey a range from 3.8 to 7.2 in adsorbing the same amount of base.

The fact that the curves originate at different points on the ordinate axis and the fact that they all intersect make it very obvious that the exchange equivalent weights of different soils may be very different in their relative magnitudes at different pH values. Thus the ratio 8 Nipe, 5 Sassafras, and 1 Sharkey holds for pH 7.0 only. At a higher pH the ratio will be narrower, whereas at a low pH it will be wider. At a pH well below 6.0 where the Nipe combines with and exchanges practically no cations this ratio approaches infinity. The reason for this difference is, as already stated, the differences in the strength and magnitude of the acid residue. The Nipe laterite may possess a very large acid residue but it is too weak to become engaged except at higher pH. The sesquioxides react as acids only at a high pH. The Nipe contains very little acid residue active below pH 7, such as silicic and humic acids. Titanium oxide is isoelectric at about pH 4.8 and binds a considerable amount of base at 7 but in most cases TiO_2 makes up only from 0.5 to 1 per cent of the soil colloid complex. (The amphoteric titanium series are now being studied together with similar series of other elements in the periodic system. The results will be published shortly.)

SATURATION, ALUMINUM SOLUBILITY, AND PLANT INJURY IN RELATION TO THE ISOELECTRIC POINT

The recognition of the amphoteric nature of the soil complex will prove very useful to soil chemistry. It will enable us to account for many phenomena which the one-sided conception of the soil complex as an acidoid could not do. Thus the "influence" of the anions upon the displacement of H ions by the neutral salt cations is easily accounted for when we know that the anions themselves enter into combination with the complex in increasing numbers as the pH is lowered, all depending upon the nature of the ions and upon the strength of the basic residue. Again, it will be readily understood why there can be no simple relationship between the pH and the degree of base saturation as would be expected if the soil complex represented a single, definite "soil acid."

Another phenomenom which has a bearing on the point of injury to plant growth may here be mentioned. Pierre (6) has made the important observation that the higher the percentage base saturation at a given pH the lower is the aluminum content in the solution. The obvious explanation of this is that the higher the base saturation at a given (low) pH the lower is the isoelectric point of the complex. This means a weak and less reactive basic residue, which requires a higher H-ion concentration for its ionization and solution, by hydrolytic cleavage, into mobile aluminum ions (single or complex). A low base saturation, at the same given pH, means, on the other hand, a high isoelectric point, a stronger and more active basic residue, and therefore a greater ionization and mobilization of aluminum.

The relationship is clearly shown by the three soils here studied. When the electrodialyzed soils (zero saturation) are treated with a neutral salt the aluminum in solution decreases in the following order:

Sharkey > Sassafras > Nipe

The exchange acidity is so much greater in the Sharkey that, although the complex of this soil not only contains less aluminum but holds it in a less active condition due to the strong acid residue [at a given pH the Sharkey colloid adsorbs by far the smallest quantities of neutral salt anions (3, VI)], more aluminum passes into solution from this soil than from the other two whose exchange acidity is much lower (comp. fig. 28). But if the pH of the Sassafras and the Nipe be brought down to that of the Sharkey by the addition of acid the order of the aluminum and iron solubility reverses itself and becomes

Nipe > Sassafras > Sharkey

exactly as the theory demands.

It is quite obvious that any attempt to relate the pH to aluminum solubility and plant injury must meet with failure until proper account is taken of the amphoteric nature of the soil studied. At a given low pH the Nipe soil would undoubtedly be the most toxic to plants, as it would then be on the acid and

electropositive side of its isoelectric point and would therefore yield a greater concentration of aluminum. But it must not be forgotten that the Nipe soil (laterite) would most strongly resist a lowering of the pH. Except under abnormal conditions this soil would always maintain a higher pH in the unsaturated condition. In fact, the ultimate pH of this type of soils is often so high that it is doubtful whether aluminum would ever, under normal conditions, become soluble to the extent of causing injury to plants. Manganese appears to be a greater source of danger in these soils, but this question will be dealt with later.

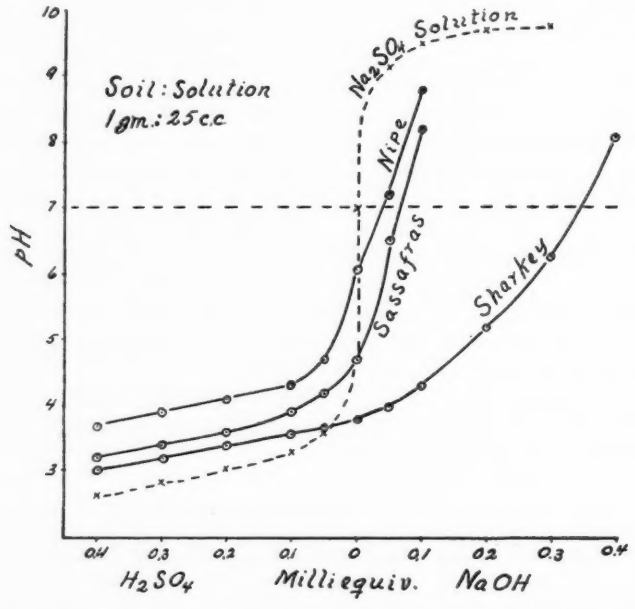

FIG. 28. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY OF THREE DIFFERENT ELECTRODIALYZED SOILS TO BIND ACID AND BASE

THE AMPHOTERIC REACTIONS OF PODZOLIZED SOILS

Very briefly stated, a podzolized soil is characterized by the following morphological and chemical differences (1):

The A_0 layer, or the uppermost layer, consisting of organic matter representing every stage of transformation. The humic material of this layer is usually very acid.

The A_1 horizon is the mineral horizon immediately below the A_0 layer. It contains a certain amount of humic materials and has a low pH.

The A_2 horizon when well developed is recognized by its ash-gray or bleached appearance. Its pH is then also low. The colloidal content of this horizon is relatively low. The mineral A horizons represent the zone of eluviation. From this zone certain materials, notably the

sesquioxides but also silicic and humic acids as well as strong bases, move downward in the dissolved or highly dispersed condition, only to be precipitated and accumulate at a greater depth.

The B horizon represents the horizon of accumulation or illuviation and is recognized by a greater compactness and a brownish color. The pH in this horizon is markedly higher than that of the preceding horizons. Its colloidal content is also relatively high.

The C horizon is represented by the underlying parent material. The pH in this horizon is usually the highest.

These changes within the podzolized soil body must primarily be ascribed to the prevailing pH in the various horizons. The pH is in turn conditioned by

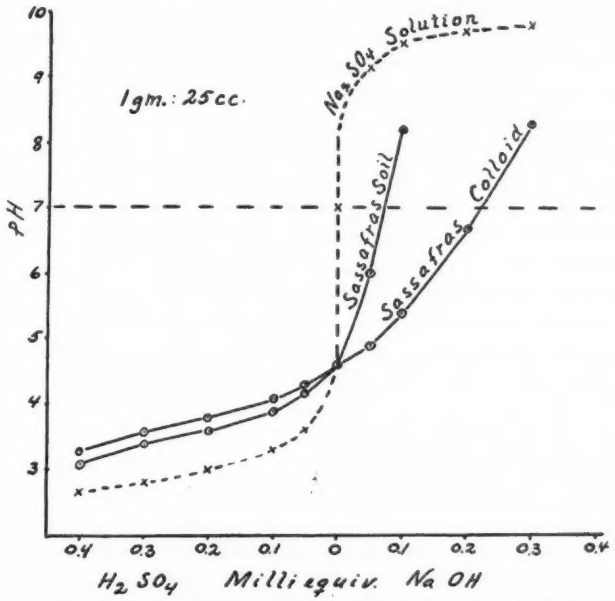

FIG. 29. THE pH OF EXCHANGE NEUTRALITY AND COMBINING CAPACITY OF THE ELECTRODIALYZED SASSAFRAS SOIL AND COLLOID

the climate and vegetation, which give rise to the accumulation of the sour, humic layer. A low temperature and a heavy rainfall together with a vegetation having a low ash content favor the formation of such a layer. The characteristic bleached podzol horizon is the result of an acid hydrolysis of the colloidal complex which follows an extensive leaching and unsaturation of the complex. This acid hydrolysis leads to an ionization of the basic sesquioxide constituent of the complex. Iron and aluminum move downward, chiefly in the form of ionic complexes, in union with organic (humic), silicic, and other acids. Aluminum and iron in "solution" at a pH of about 4.0 cannot exist as Al^{+++} and Fe^{+++} ions. Combinations like $Al^{+}OH\ A$, $Al^{+}\ A_2$, etc. (where A = acid

anion) are certain to exist. These ionic complexes are precipitated in the B horizon where the pH is higher and the complex therefore more nearly isoelectric. The precipitation may be visualized as follows:

$$Al^{+} A_2 + OH^{-} \rightarrow OH\ Al\ A_2$$

On the basis of the theory of isoelectric weathering, as outlined in the preceding part (3, IX) of this series, the composition of the amphoteric complex must be governed by the prevailing pH in such a way that the higher the pH the greater must be the basic, in this case, the sesquioxide proportion, in the complex; that is, the acidoid/basoid ratio must be low. A low pH, on the other hand, must lead to the formation of a complex in which this ratio is high. This is because the isoelectric pH, i.e., the pH of maximum stability, increases with the basoid or sesquioxide content and vice versa (3, III).

If this were so, then the colloidal complex left behind in the very acid A horizon should possess a high silica/sesquioxide ratio (acidoid/basoid ratio in general) and should yield relatively low amphoteric points. That is, the isoelectric pH, the pH of exchange neutrality and the ultimate pH should all be low. Such a complex can alone be stable in this horizon. The isoelectric point of the original complex (before podzolization) would be too high for the new, more acid, environment. By losing some of its sesquioxide component by the acid hydrolysis the complex automatically adjusts itself into a condition of stability.

The colloidal complex accumulating and built up in the B horizon where the prevailing pH is higher must, to be stable, assume a relatively lower acidoid/basoid ratio (since humic acids partly enter in place of silicic acid in this complex it is more rational to employ the general term for this ratio). The isoelectric point, the pH of exchange neutrality, and the ultimate pH must therefore be correspondingly higher in the B horizon as compared to the same values in the A horizons. The following investigation will show how well the theory fits the facts.

We selected for our study a sandy soil of the Lakewood series in the vicinity of Cedar Bridge, New Jersey. How well the morphological characteristics of the podzol profile have developed in this soil is shown in plate 2.

The area from which this profile was taken supported a forest growth predominantly oak with some pine mixed in, the average age being estimated at about 25 years. The shrubby and herbaceous ground cover was mostly ericaceous. There was ample evidence of recurring forest fires, and the general appearance of the stand indicated poor growth.

Table 95 shows the percentage of carbon and the pH of the original soil in the various horizons.[2] The table also gives the percentage of the major constituents and the silica/sesquioxide ratio of the extracted colloids. The latter were extracted by the supercentrifuge after dispersion in a slightly alkaline solution of Na_2CO_3

[2] For the information contained in the table we are indebted to Dr. C. Watson who has made a separate study of this soil.

It will be seen that the pH increases downward from a value of 4.0 in the A_1 to 5.15 in the C horizon. The low pH in the A horizons has led to an extensive acid hydrolysis of the complex as indicated by the remarkably high silica/ses-

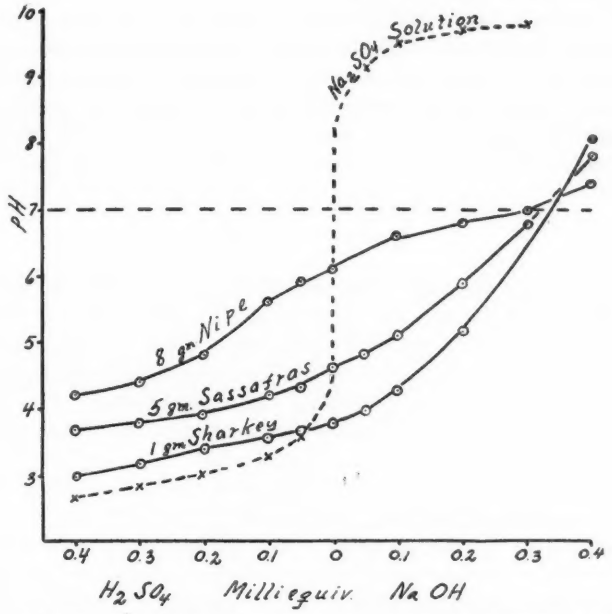

FIG. 30. THE NEUTRALIZATION CURVES OF THE THREE ELECTRODIALYZED SOILS IN FIGURE 28 WHEN TAKEN IN QUANTITIES OF EQUIVALENT BASE-BINDING POWER AT pH 7

TABLE 95

The pH and carbon content of a Lakewood podzol and the major constituents of the extracted colloids

HORIZON	WHOLE SOIL		EXTRACTED COLLOIDS			
	pH	Carbon	SiO_2	Al_2O_3	Fe_2O_3	$\frac{SiO_2}{R_2O_3}$
		per cent	*per cent*	*per cent*	*per cent*	
A_1	4.0	3.70	74.00	14.02	2.23	8.11
A_2	4.2	0.27	79.40	14.09	2.06	8.78
B	4.65	1.23	45.40	35.81	11.69	1.78
C	5.15	0.09	45.60	21.67	10.15	2.74

quioxide ratios. This ratio is lowest in the B horizon where the sesquioxides have accumulated. The fact that the silica/sesquioxide ratio is higher in the C than in the B horizon although the pH is highest in the former must be due to the immature condition of the complex in the C horizon. As long as the

complex remains saturated to an appreciable extent with divalent cations, silica will be retained in excess of that quantity which is held by the sesquioxides alone. All unleached soil colloids possess a high silica content. The fact that the complex in B contains more humic acid in place of silica than the complex in the C horizon must also be considered.

The percentage of carbon indicates a high organic matter or humus content in the A_1 horizon. It is also fairly high in the B horizon. The quantity of humic acids present in the complex is important when we consider the relationship between the silica/sesquioxide ratio and the amphoteric behavior of the complex. On the basis of this ratio the isoelectric pH should be in the following descending order

$$B > C > A_1 > A_2$$

but this order may obviously be modified by the presence of humic acid or any other acidoid and basoid. The silica/sesquioxide ratio does not then, even approximately, express the acidoid basoid ratio. Then it must again be emphasized that the ratio of silica to sesquioxides is a mass ratio and does not, therefore, express the activity of the acidoid and basoid residues. This will vary with the relative proportions between aluminum and iron, with the degree of hydrolysis, and with the dispersion of the material.

The chemical composition of the colloid does not in itself supply us with any adequate information concerning the nature of the complex.

Let us now see how the soil materials from the various horizons react. Figure 31 shows the neutralization curves of samples from the four horizons. Because of the sandy nature of the soil 10 gm. was taken in each case.

It will be noted that the samples from the A horizons whose colloids contain over 8 moles of silica to each mol of sesquioxide do not react amphoterically to any definite degree. The slight exchange alkalinity developed by A_1 at very low pH may be ascribed to the protein fraction of the organic matter in this material. The materials of the B and C horizons react, in strong contrast to that of the A horizons, definitely amphoteric. The pH of exchange neutrality in the Na_2SO_4 solution is 5.0 in the B and 4.8 in the C horizon. The strength of the acid residue in the different complexes assumes the following order:

$$A_1 > A_2 > C > B$$

This is in agreement with the silica/sesquioxide ratios except in respect to the position of A_1 and A_2. This discrepancy is of course largely due to the much higher humic content in the A_1 horizon.

The influence of the humus expresses itself quantitatively more strongly than qualitatively, as will be seen by comparing the combining capacity of the A_1 and A_2 samples. Humic acids are no stronger acids than many of the mineral complexes having a high silica content (3, VI), but the combining capacity for bases is, because of the relatively small equivalent weight of humic acids,

considerably greater as compared to the mineral complex. Whereas 10 gm. of the A_2 sample binds only about 0.15 m.e. NaOH at pH 7.0 we find by extrapolation that the A_1 sample binds about 0.8 m.e. of the base.

The small combining capacity of the A_2 sample as compared to the B and C samples is of course due to a smaller colloid content of the A_2 horizon. The combining capacity per gram colloid would undoubtedly be greater in A_2 than in B or C, since the cation exchange increases, within certain limits, with the silica sesquioxide ratio (3, V). Whereas the combining capacity of a soil sample varies with the colloid content the pH of exchange neutrality remains

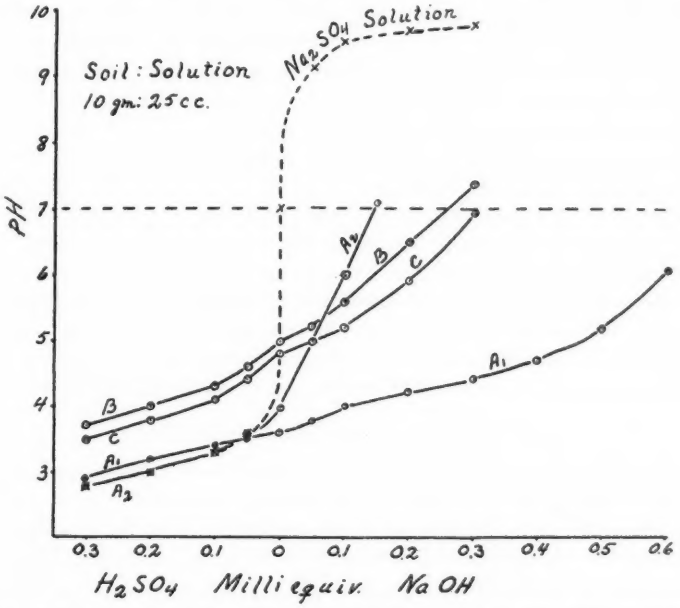

FIG. 31. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF A LAKEWOOD PODZOL PROFILE

unaffected, at least within wide limits. This is extremely important because it enables us not only to determine, by a very simple test, the amphoteric nature of the complex, but also, by comparing the exchange reactions of samples from the different horizons, to determine whether or not a soil is being podzolized.

The acid-oxalate extraction method proposed by Tamm for the study of podzolization has proved very useful (7, 2) but any method involving a chemical analysis is too time-consuming to be generally adopted. Furthermore, any method which is based upon the solvent action of a certain solution must always

remain more or less empirical. There are many other methods by which evidence of podzolization may be obtained. Some of these have recently been discussed by Joffe (1).

Since the soil colloidal complex reacts amphoterically and since the chief result of podzolization consists in a change in the amphoteric nature of the complex according to a well-defined order, namely, a more acidic and less basic A horizon and a less acidic and more basic B horizon, it would seem that the most rational method is to be found in a determination of the amphoteric points of the respective complexes. The isoelectric points may be found cataphoretically, but this requires a costly apparatus. A determination of the ultimate pH or the pH of exchange neutrality is much more simple where no apparatus is available. Either method will yield the information sought. In the latter case, however, it is necessary that the soils be completely unsaturated, by electrodialysis or otherwise. The pH of the soil in water represents then the ultimate pH whereas its pH in a neutral salt solution represents, very nearly, that pH at which the anions and cations combine with the soil complex in equal numbers.

In this work a normal Na_2SO_4 solution was employed. It is possible that another salt and another concentration will prove more suitable. It is desirable to reduce the salt effect to a minimum, in view of which a more dilute solution might be chosen.

If the isoelectric point is to be determined by cataphoresis it is recommended that the pH be adjusted by HCl and that the soil be suspended in 0.01*N*, or less, NaCl. The presence of the salt greatly hastens the equilibrium so that the measurements can be made without serious errors the day after mixing.

THE REACTION OF AMPHOTERIC PRECIPITATES

In the following we shall present the neutralization curves of a number of the isoelectric precipitates discussed in the previous parts of this series (3, IX). The precipitates were prepared in large quantities in solutions less dilute than those used for the cataphoresis experiments referred to. They were then dried in a warm place, washed on the filter, and finally electrodialyzed, dried, and analyzed. The composition is given in the figures.

Aluminum and ferric "hydroxides" and "silicates"

Figure 32 shows the neutralization curves and, at the points of intersection with the salt solution curve, the pH of exchange neutrality of aluminum "hydroxide" of three "silicates" of increasing degree of silication and of silica gel. Figure 33 gives the same values for the ferric complexes.

The figures require but little comment. Those who have read the preceding articles on isoelectric precipitates will recognize the same laws of amphoteric behavior: the displacement of the pH of exchange neutrality (as in the case of the isoelectric point) to the acid side and the increase in the cation (base) combining capacity as the silication of the complex is increased. Simultaneously

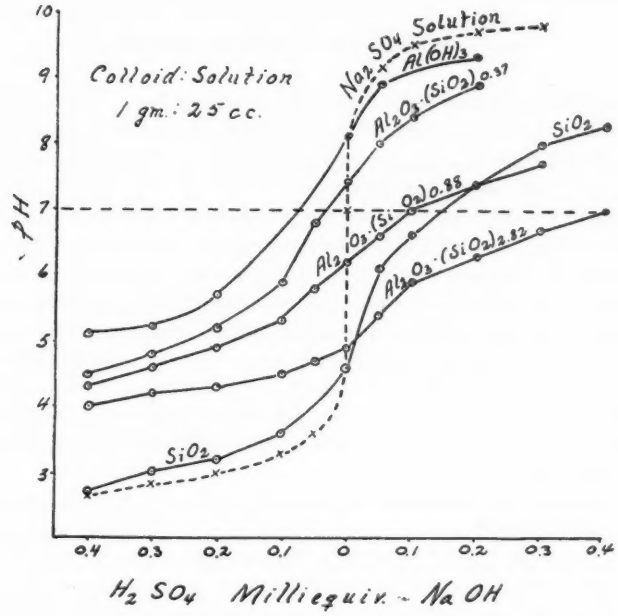

FIG. 32. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF ELECTRODIALYZED ALUMINUM HYDROXIDE, SILICA GEL AND THREE ALUMINUM "SILICATES"

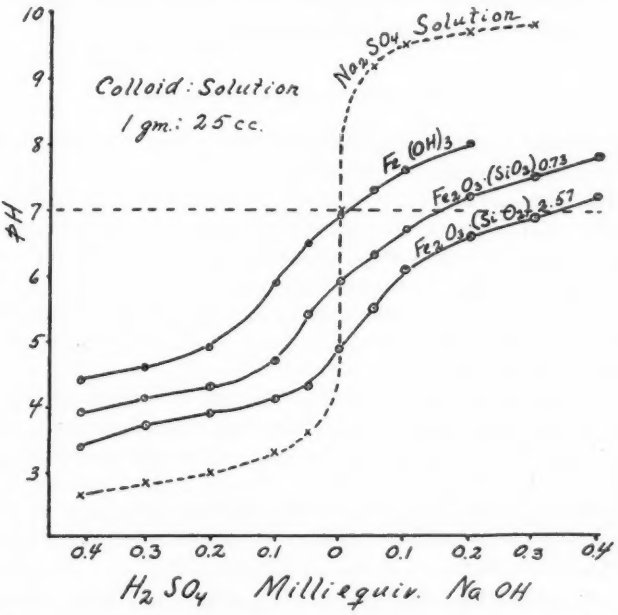

FIG. 33. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF ELECTRODIALYZED FERRIC HYDROXIDE AND TWO FERRIC "SILICATES"

the complex loses its power to combine with anions (acids). The entrance of silicic acid into the complex strengthens the acid and weakens the basic residue. The fact that alumina is a stronger base than ferric oxide is brought out by the relative position of the amphoteric points.

The case of the silica gel is interesting in that it shows a lower cation combining power than the most highly silicated complex. This shows that the silicated sesquioxide complexes must attain a maximum in respect to the power to bind bases. This maximum has now been found and will be discussed in a later publication. The fact that the curve of the silica gel intersects the salt solution curve and thereby indicates a power to bind acid cannot be explained unless the basic ions were incompletely removed by electrodialysis. Silica may be amphoteric and bind acids at a very low pH but an exchange neutrality at pH 4.6 in Na_2SO_4 would be highly anomalous in view of the behavior of the higher silicate complexes.

It will be noted that aluminum "hydroxide" together with the least silicated complex, both of which are stronger as bases than as acids and are therefore isoelectric above pH 7, yield an actual exchange alkalinity with the neutral salt solution just as the complexes whose acid residue is the strongest yield an actual exchange acidity. But even the latter type of substances yield, below the pH of exchange neutrality, an exchange alkalinity inasmuch as they cause a rise in pH. In a broad sense the term "exchange alkalinity" expresses therefore a rise in pH whereas the term "exchange acidity" expresses a lowering of the pH, no matter what side of the neutral point is involved.

The aluminum and ferric phosphates

Figures 34 and 35 show the exchange-neutral points and the combining capacity of a few phosphated sesquioxides.

What has been said about the silicated complexes applies in general to the phosphated. But there are noteworthy differences. Thus the phosphate ion lowers the amphoteric points more, increases the cation combining capacity more, and suppresses the anion combining capacity more than the silicate ion. In other words, the phosphate ion leaves a relatively stronger acid residue and a weaker basic residue than the silicate ion. This is all in agreement with earlier observations (3, III).

If we compare the capacity of these precipitates to combine with bases as indicated by their neutralization curves, with their cation exchange capacity as reported in part V (3) it will be noted that the latter are appreciably greater. Although this might in part be ascribed to the use, in these experiments, of a more highly dissociable cation (Na), which therefore possesses a weaker displacing power, it is perhaps primarily due to the aged condition of the gels here used. The gels might have undergone considerable changes. Compounds of greater stability and lower reactivity might gradually be formed and crystal-

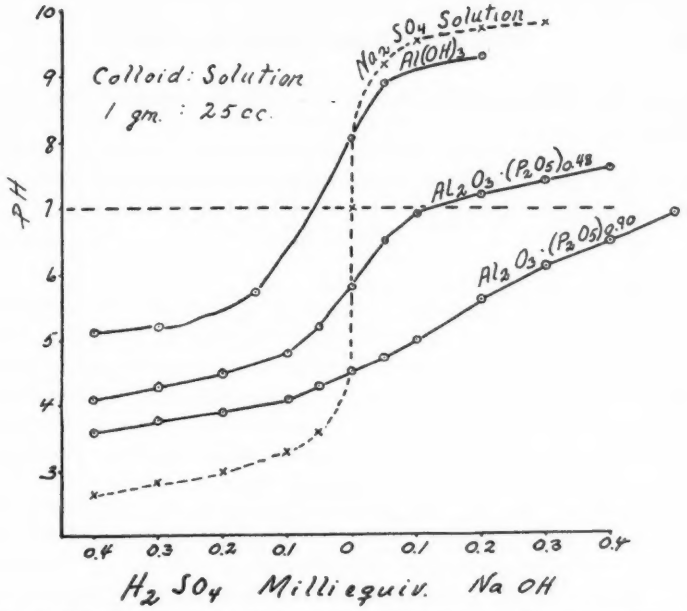

FIG. 34. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF TWO ELECTRODIALYZED ALUMINUM "PHOSPHATES" AND OF $Al(OH)_3$

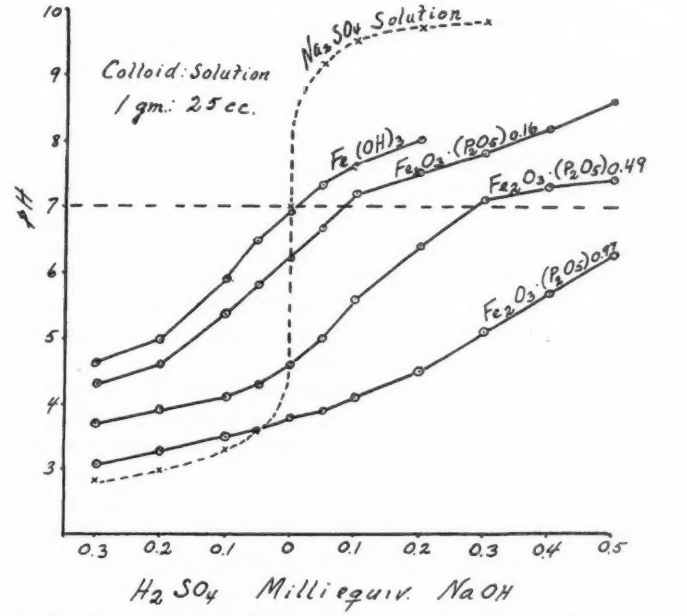

FIG. 35. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF THREE ELECTRODIALYZED FERRIC "PHOSPHATES" AND OF $Fe(OH)_3$

lized. The following might serve to illustrate the general nature of such a change:

$$(OH)_2\,B{-}A\,H_2 \rightarrow OH\,B = A\,H + H_2O$$

or:

$$OH\,B = A\,H \rightarrow B \equiv A + H_2O$$

all depending upon the relative stability.

The degree of the combination will depend upon the pH. At the isoelectric point where the ionization of the ampholytoid is at a minimum, there will be a maximum combination between the acidoid and the basoid constituents. Above and below this point there will be increasing hydrolysis resulting in a stronger acid and basic residue. Hence the combining power or exchange capacity of the colloid will be "built up" on the alkaline or acid side. The formation of more stable and less reactive compounds would therefore be favored by the electrodialyzed, that is, by the uncombined, condition of the ampholytoid. In this way we might account for the loss of combining power of the electrodialyzed and aged gels used in these experiments. Kaolin and other inactive and coarsely crystalline compounds may be assumed to be formed under conditions of unsaturation and a prevailing pH near that of the isoelectric point of the complex.

Aluminum and ferric "humates"

Figure 36 shows the exchange neutral points and the combining capacity of aluminum and ferric "humates" and of humic acid. The figures in the formulas $Al_2O_3 \cdot (Hum)_{0.265}$ and $Fe_2O_3 \cdot (Hum)_{0.277}$ express the gram weight of humic acid per millimol sesquioxide. These materials were prepared according to system 54 and 55 as shown in part IV (3). The "humates" were here used in the dry, powdered condition. Because of the irreversible, nondispersible nature of these materials, a considerable loss in combining power has resulted from the drying. The humic acid was used in the moist suspended form in which it has been kept. Because of the very much greater combining power of the latter only 0.25 gm. was taken for this experiment whereas the usual 1-gm. sample was taken in the case of each of the "humates."

The isoelectric pH values of the aluminum and ferric "humates" were 5.5 and 4.55 respectively. The pH of exchange neutrality in Na_2SO_4, as shown in figure 36, is 5.8 and 4.7 respectively. In spite of the considerable loss in combining power (see part V) the relative activity of the acid and basic residue seems therefore to have maintained itself. The capacity to bind base is also consistently greater in the ferric than in the aluminum complex. The ferric hydroxide, being a weaker base, leaves a greater as well as a more active acid residue.

Humic acid, as already noted (3, VI) reacts amphoterically but its basic

group is weak and comes into play only at a low pH. Its pH of exchange neutrality in Na_2SO_4 is about 3.6. The amphoteric nature of humic acid can be ascribed to the amino groups of the protein fraction of the complex.

Because of dispersion the humic acid curve could not be extended to the higher pH by the indicator method which was uniformly employed to compensate as much as possible for the salt effect.

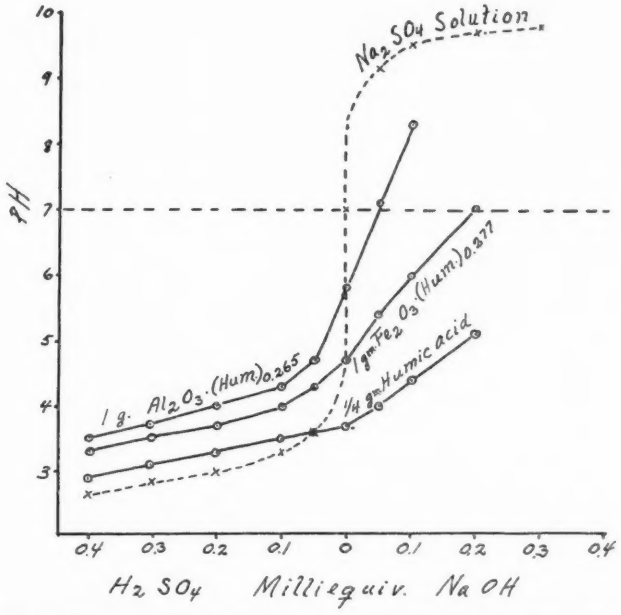

FIG. 36. THE pH OF EXCHANGE NEUTRALITY AND THE COMBINING CAPACITY OF ELECTRODIALYZED ALUMINUM AND FERRIC "HUMATES" AND OF "HUMIC ACID"
Figures express gram humus per millimol sesquioxide

Protein and protein "humates"

The behavior of protein "humates" has already been discussed (3, VII). Figure 37 shows the neutralization curves of egg albumin, of humic acid, and of a mixture of both, each in 0.25-gm. quantities. The albumin was not electrodialyzed. The point of intersection with the salt solution curve has therefore no definite relation to the isoelectric point.

The point of interest is the general form and position of the curves. The albumin combines with much more acid than the humic complex whereas the power to bind base is far greater in the latter. In a mixture of the two substances there results a complex which partly combines the properties of both.

The fact that the mixture, that is, albumin "humate," binds less acid than albumin alone and that, at the lower pH, it binds less base than the humic acid alone, proves that a mutual neutralization has taken place. At pH below the iso-electric point (about 4.8) of the albumin, where it plays the part of a base, it combines with humic acid forming a complex whose isoelectric point and combining capacity are intermediate to those of the individual components (3, VII). At pH above its isoelectric point the albumin combines primarily as an acid and must here add to the base binding power of the mixture. At high pH, the curve representing the mixture would undoubtedly cross the humic acid curve as indicated by the dotted extension of the curves.

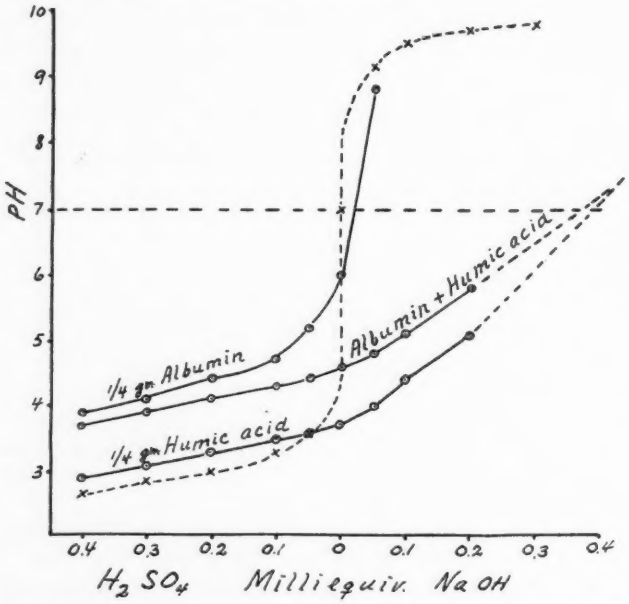

FIG. 37. THE NEUTRALIZATION CURVES OF ALBUMIN, OF "HUMIC ACID," AND OF ALBUMIN "HUMATE"

A CHEMICAL CLASSIFICATION OF SOILS

The most fundamental numerical values of acids and bases express their strength as defined by their dissociation constant and their combining capacity or equivalent weight. Substances which combine the properties of both acids and bases are in addition characterized by their isoelectric point. Since soil colloids react amphoterically and possess, in various degrees, the properties of acids and bases it would seem that the most logical and scientific manner in which chemically to characterize and classify soils would be found in a numerical expression of their strength and capacity to react.

As already pointed out in part VI of this series (3), we cannot express the strength of a colloidal acid or base in the same terms, i.e., by the dissociation constant, by which we define the strength of the soluble acids and bases because we cannot apply the mass law to colloidal ionogens. But values such as the ultimate pH, the isoelectric pH, and the pH of exchange neutrality are all expressions of the strength of the acid and basic residues and, although these terms are no absolute measure of strength, they can be used and relied upon on a relative scale.

Since the ultimate pH and the pH of exchange neutrality represent the activity of the free, unsaturated ampholytoid (or acidoid) it is of course necessary that the soil be electrodialyzed or otherwise unsaturated before these values are determined. The ultimate pH of a soil might be written pH_U whereas the pH of exchange neutrality might be designated $pH_{U(KCl)}$ the formula within parenthesis denoting the salt solution employed. The U would distinguish these pH values from the corresponding values of the original, untreated soil.

When expressing the combining capacity or equivalent of a soil it is obviously necessary to state the pH at which the determination was made. Colloids contain displaceable H and OH of widely differing activity. As examples of very weak acids $M''HPO_4$ and glucose may be mentioned, which both yield an acid dissociation constant as low as 3.6×10^{-13} (5). The H ions of these and other acids, of the same order, may not even begin to become displaced at a pH from 7 to 8.5, the usual upper limit in soils. (The degree of displacement at any pH will of course depend upon the dissociation of the salt formed.)

Very weakly acidic, basic, or amphoteric properties may be very generally associated with supposedly inert materials. It is conceivable that such properties depend entirely on the way a substance reacts with water, which contains the elements of both acids and bases. Cellulose is acidic as shown by its exchange reaction with KCl. The acidic nature of colloids appears to predominate. Michaelis (4) states that colloids divide themselves into acidoids and ampholytoids. No basoids are known. But it is only those acidoids which are active enough to become engaged within the usual range of soil reaction which are of importance in relation to the cation combining power of a soil. These acidoids comprise silicic acid and the silicated, humic acids and the humated, the proteins and the proteinated, and the phosphated complexes. To this list we might add titanium oxide. The sesquioxides are too weakly acidic appreciably to combine with bases below the usual maximum of soil pH. At high alkalinity a number of very weak acid groups come into play in addition to other stronger groups, set free by a hydrolytic cleavage of the complex. The "total" exchange capacity is therefore an indeterminable quantity.

As a measure of the base combining or exchange capacity of soils the amount of Ca adsorbed in equilibrium with $CaCO_3$ would seem to be the most natural value. This value could also be obtained by carrying the neutralization to the corresponding pH. The important point is that soil investigators all agree upon a certain pH value and procedure for determining the combining capacity.

This capacity might be expressed by the symbols $C_{(7.0)}$, $C_{(8.0)}$, or $C_{(CaCO_3)}$, the figures denoting the pH at which the capacity is determined or that the capacity was determined in equilibrium with $CaCO_3$.

The determination of the aforementioned values serves to define the soil in terms of its chemical character, qualitatively as well as quantitatively. Because of their intimate relationship to most of the properties of the soil, these values yield, in addition, a very general, if indirect, information about the soil and enable us, in a large measure, to interpret and to predict its behavior.

In addition to these values which must be looked upon as fundamental and, for long periods of time, fairly constant, we have to consider another value which is more subject to variation and which expresses the condition or degree of saturation of the soil complex. This value can be expressed numerically only with reference to a certain C value or to Hissink's T value. The degree of saturation or unsaturation need not be determined by a separate analytical method, as it is usually done, but may quite simply be computed from the neutralization curve of the electrodialyzed soil as given in figures 28 to 31. The only additional information required is the pH of the soil in its original condition. The neutralization curve gives the combining capacity of the soil at any pH.

Figure 28 shows the combining capacities of the Sharkey soil (for Na ions in the presence of N Na_2SO_4) to be $C_{(4.3)} = 0.1$, $C_{(5.2)} = 0.2$, $C_{(6.3)} = 0.3$, $C_{(7.0)} = 0.345$ and $C_{(8.0)} = 0.395$ m.e. per gram.

Assume now that the pH of the Sharkey soil is 6.3 in its natural condition. At this pH it would contain 0.3 m.e. exchangeable cations (we are here ignoring any errors resulting from a radically different set of conditions). At pH 7.0 the soil would contain 0.345 and at pH 8.0 it would contain 0.395 m.e. of cations other than H ions. The percentage saturation would be

$$\frac{C_{(6.3)} \times 100}{C_{(7.0)}} = \frac{0.3 \times 100}{0.345} = 87 \text{ per cent}$$

with reference to the combining capacity of the soil at pH 7.0, and

$$\frac{C_{(6.3)} \times 100}{C_{(8.0)}} = \frac{0.3 \times 100}{0.395} = 76 \text{ per cent}$$

with reference to a pH of 8.0.

Since most of the exchangeable cations consist of Ca ions, the most comparable results would be obtained by using $Ca(OH)_2 + Ca\ Cl_2$ instead of sodium. A thorough-going investigation would be desirable in order to determine the procedure most nearly applicable to natural conditions.

We thus see that practically all the inportant information dealing with the chemical nature of the soil may be obtained by the comparatively simple method of plotting the neutralization curve of the soil in its unsaturated condition. In a later publication we shall discuss the relationship between the am-

photeric nature of soils, as here brought out, and the point of injury to plant growth.

SUMMARY

The amphoteric nature of soils and their combining capacity are expressed in the form of neutralization curves obtained by treating the electrodialyzed soil with a neutral salt solution to which free acid is added on the one side and free base on the other.

The pH developed in the neutral salt solution represents very nearly the pH of exchange neutrality, that is, the point at which the soil combines with an equal number of anions and cations of the salt. This point is related to the strength of the acid and basic residues of the soil complex. The ultimate pH is defined as the pH of the completely unsaturated soil ampholytoid. This also is strictly an intensity factor.

The combining capacity for anions and cations depends upon the quantity of the acid and basic residues, sufficiently active to become engaged at the pH in question.

Evidence of podzolization, which involves an alteration of the amphoteric complex by hydrolysis, may be found by applying the method to the soil profile.

The colloid content and the saturation value may also be directly found from this type of curve.

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PLATE 2

THE LAKEWOOD PODZOL PROFILE FROM WHICH THE SAMPLES WHOSE NEUTRALIZATION CURVES SHOWN IN FIGURE 31 WERE TAKEN

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